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CONTENTS OF VOL. III.

No. 1. January, 1929.

No. 2. April, 1929.

CONTENTS

No. 3. July, 1929.

No. 4. October, 1929.

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STUDIES ON DENATURATION OF PROTEINS

VII. DENATURATION VERSUS COAGULATION

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Hardy (4) in 1899 put forth the view that heat coagulation of proteins takes place in two stages, the first of which, denaturation, is a preliminary change in the chemical structure of the protein, while the second, flocculation, is a purely physical process. This view was adopted and amplified by Chick and Martin (2) who defined denaturation as the reaction between the protein and hot water and used the term agglutination to designate the process of the separation of the denatured protein in a particulate form. That the protein can be separately denatured and flocculated has been confirmed by others, and the composite nature of coagulation seems to be generally accepted.

According to this view heat coagulation is nothing more than denaturation followed by flocculation (or agglutination). Since flocculation is physical, it has been customary in previous investigations, including those of ourselves, to regard denaturation and coagulation as one and the same process as far as any chemical change is concerned. Consequently, conclusions regarding denaturation have been drawn from experiments primarily on coagulation, and vice versa. This has led to two theories of heat coagulation (or denaturation) of protein which are diametrically opposite to each other. According to one theory, denaturation (and therefore coagulation) is a mild hydrolysis or degradation; (5, 11, 12) according to the other, coagulation (and therefore denaturation) is a dehydration or condensation (7, 8). The chemical identity of the coagulated protein with the denatured and flocculated protein does not seem to have been questioned.

If egg albumin is first heated in a slightly acid or alkaline solution so that the denatured albumin remains in solutlon, and then the solution is cooled and neutralized, the protein which flocculates is readily redissolved by a slight excess of acid or alkali. If, however, the egg albumin is heated in a solution of such hydrogen ion concentration that flocculation occurs during the heating, the product obtained is practically insoluble in dilute acids and alkalies. Evidently, then, there is a difference between the albumin which has been separately denatured and flocculated and that which has been coagulated in one operation. For the sake of brevity we will call the former flocculated albumin in this paper. Chick and Martin (3) also recognized the difference between the flocculated and the coagulated albumins, but they were inclined to the view that they represent different forms of the same chemical individual. They believed that the different degrees of cohesion of the particles would account for the difference in solubility. This view does not seem to be correct, since the difference in solubility between the flocculated and the coagulated egg albumins is not merely quantitative but qualitative. The coagulated protein will not dissolve, even on long standing, in such solutions of dilute acids or alkalies as will just completely dissolve the flocculated protein.

There is also a difference in physical appearance between the flocculated and the coagulated egg albumin. The former is fluffy and more or less transparent while the latter is granular and opaque. If the flocculated albumin is heated, it loses its solubility in dilute acids and alkalies and becomes indistinguishable in physical appearance from that of the coagulated albumin.

We have recently observed another difference between flocculated albumin and coagulated albumin in the course of a study of their molecular weight.* Burk and Greenberg (1) have shown that concentrated urea solutions exert a powerful solvent action on proteins which are insoluble in water at the isoelectric point, and they have suggested the use of this solvent for the determination of molecular weight of proteins. In applying this method to flocculated and coagulated egg albumins we found the former to be soluble, while the latter insoluble, in 40 per cent urea solution. Flocculated albumin after heating in the isoelectric region also becomes insoluble. Albumin which has been denatured by alcohol and then flocculated by neutralization is soluble, but that coagulated by alcohol is insoluble. Albumin coagulated

*This work is in progress and will be reported later.

by shaking is also insoluble. There is thus a sharp line of demarkation between the flocculated and the coagulated egg albumins with regard to solubility in concentrated urea solution.

The difference between flocculated and coagulated proteins is not always so marked as in the case of egg albumin. Thus, hemoglobin coagulated by shaking dissolves readily in dilute alkalies and is somewhat soluble in concentrated urea solution. Serum albumin, coagulated by alcohol or by heat, unless the heating is prolonged, is soluble in dilute acids and alkalies and in concentrated urea solution. Coagulated proteins are, however, always less soluble than the flocculated proteins. We must conclude, therefore, that coagulation is not merely denaturation followed by flocculation, but an entirely different process.

A question of prime importance raised by the above consideration is this: Is denaturation a necessary preliminary to coagulation? In other words, can natural protein be coagulated directly without first undergoing denaturation? Since denaturation and coagulation are brought about by agents of opposite nature and under essentially opposite conditions, there is no theoretical reason to believe that denaturation is a necessary preliminary of coagulation. Experimentally, there is some evidence that coagulation can be independent of denaturation.

In heat coagulation the agent which causes coagulation also accelerates denaturation. Consequently, when a protein solution is heated for the purpose of coagulation some denaturation may also occur, unless the protein is far more susceptible to coagulation than to denaturation. However, at the isoelectric point where the rate of coagulation is at the maximum and the rate of denaturation at the minimum*, coagulation may take place without denaturation. It has been shown that denaturation of egg albumin is accompanied by an increase in acid and base binding power and the amount of this increase depends on the pH of the solution at which denaturation occurs. At the isoelectric point it cannot be experimentally determined, but by extrapolation it can be shown to be zero (9). If we assume that the change in acid and base binding power is a primary result of denatura-

*According to Lewis (6) the point of minimum denaturation coincides with the neutral point of water at the temperature of heating. According to our work reported in the following paper, the point of minimum denaturation uncomplicated by coagulation would seem to be at the isoelectric point of the protein, both points being referred to the room temperature. It should be noted that Lewis did not study denaturation alone but denaturation and coagulation together.

tion, then we may conclude that denaturation of egg albumin does not occur when it is heated at the isoelectric point for a short time. In other words, the albumin coagulated at the isoelectric point has not undergone denaturation.

That proteins can be coagulated by shaking which does not cause or accelerate denaturation (10) also supports the view that coagulation is independent of denaturation. We assumed previously that denaturation precedes coagulation by shaking. This assumption was necessary as long as coagulation was regarded as the sum of denaturation and flocculation. With the adoption of the new concept of coagulation, that assumption is no longer required.

It is interesting to record in this connection an observation on the effect of alcohol on denatured albumin. Although alcohol and shaking as well as heat can coagulate the natural egg albumin, only heat can coagulate denatured egg albumin, suspended in an isoelectric solution, while alcohol and shaking have no effect. The denatured egg albumin is thus more difficult to coagulate than the natural egg albumin. This would be contrary to our expectation if denaturation is a necessary preliminary to coagulation, but it harmonizes well with the view that denaturation and coagulation are two separate processes of opposite nature.

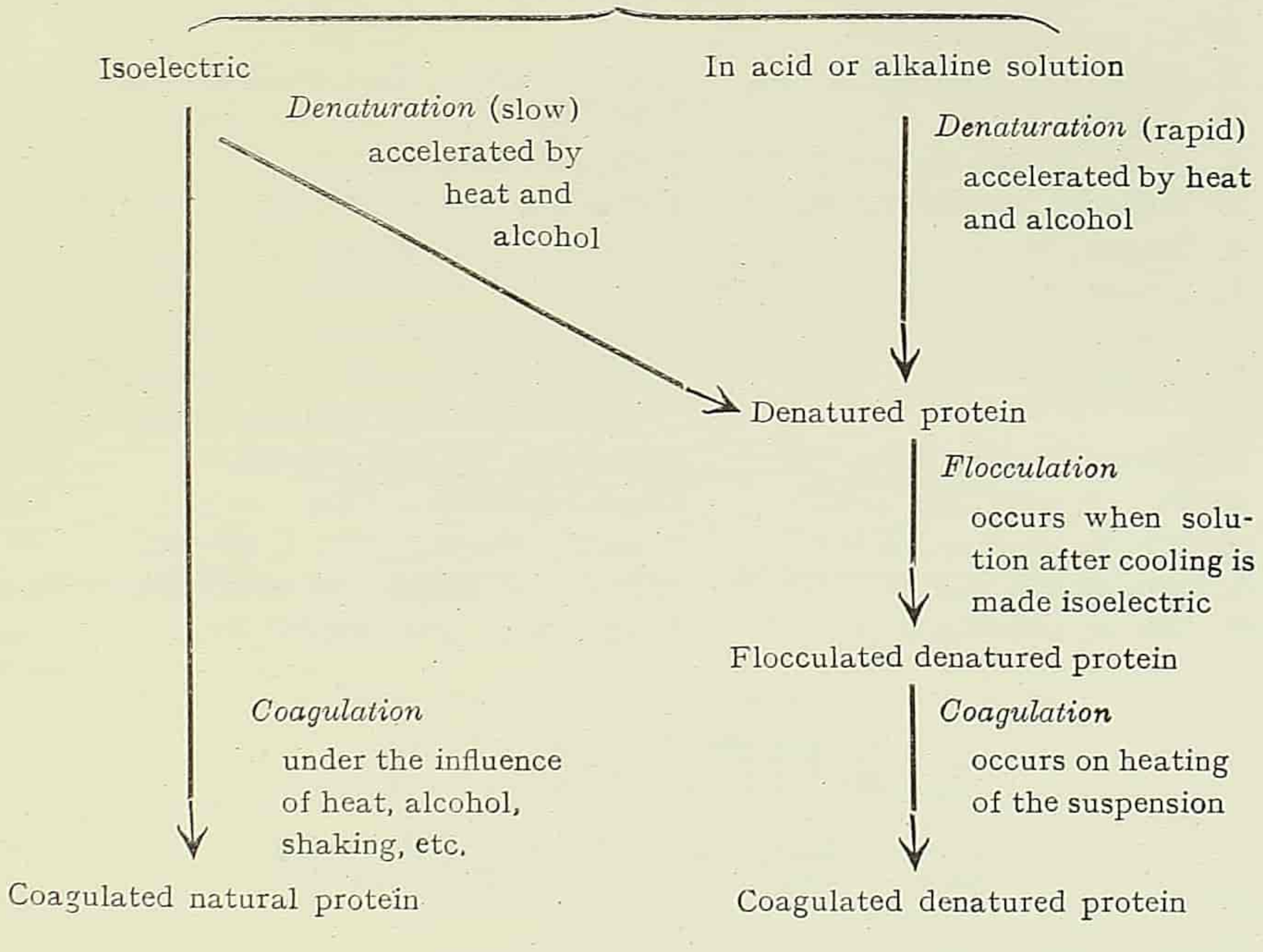
We may define denaturation, flocculation and coagulation as follows:—

(1) Denaturation is that change of the natural protein, brought about by the action of acids or alkalies, whereby it becomes insoluble in solvents in which it was previously soluble. Denaturation is slow in the isoelectric region of the protein, but it may be accelerated by heat or alcohol.

(2) Flocculation is the precipitation of the denatured protein from solution by bringing the reaction of the solution to the isoelectric region of the denatured protein. Flocculation may be compared with the precipitation of an insoluble acid or base from a solution of its soluble salt.

(3) Coagulation is that change characterized by decrease of solubility of the natural or denatured protein, brought about by the action of heat, alcohol, shaking etc., in the isoelectric region of the protein.

The relation between the three processes is shown in the following diagram.



The difference between denaturation and coagulation having been thus recognized, the apparent conflict between the two theories of denaturation disappears. The degradation theory is a theory of denaturation, while the condensation theory is one of coagulation.

SUMMARY

Evidence is adduced to show that coagulation of protein is not denaturation followed by flocculation as is generally believed, but an entirely different process.

The recognition of this fact brings into harmony the two conflicting theories of denaturation. The hydrolytic or degradation theory is a theory of denaturation, whereas the anhydride or condensation theory is a theory of coagulation.

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蛋白質之變性作用

七. 變性作用與凝固作用

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前此研究凝固作用 (coagulation) 者,咸以此爲變性作用 (denaturation) 後凝集作用 (flocculation) 之結果。本篇曾舉理由數則，以證凝固作用乃另一作用，與變性作用無關。

變性作用之性質，有謂爲分解作用 (degradation) 者,有謂爲縮合作用 (condensation) 者。二者適相反。據本篇之觀察,則二說實不衝突。蓋前者乃變性作用之學說，而後者則凝固作用之學說也。

Chinese Journal of Physiology, 1929, Vol. III, No. 1, pp. 7—28.

STUDIES ON DENATURATION OF PROTEINS

VIII. EFFECT OF DENATURATION AND COAGULATION ON ACID- AND BASE-BINDING POWER OF PROTEINS

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Received for publication August 29, 1928

The purpose of the present study is to throw light on the nature of the process underlying denaturation of proteins by ascertaining whether denatured proteins combine with more or less acid or alkali than the natural proteins. An increase in acid- and base-binding power would point to a hydrolysis or some other kind of degradation, while a decrease would indicate a change in the opposite direction.

HISTORICAL

Chick and Martin (1, 2) found that "heat denaturation" of egg albumin in alkaline solution was accompanied by a decrease of alkalinity and in acid solution by a decrease of acidity. These investigators, however, heated the egg albumin in solutions of such reaction that not only denaturation but coagulation occurred. The change in reaction which they obsevered might not be due to denaturation but to coagulation. Working with cow's hemoglobin Lewis (4) found no change of acid- or base-binding power as the result of "heat denaturation". He did not state whether the hemoglobin was coagulated or not. Judging from the conditions of his experiment and our experience with this protein, we surmise that coagulation must have occurred. Our experiments with dog's and sheep's hemoglobin have failed to corroborate his

finding. It should be noted also that he was not studying uncomplicated denaturation but rather coagulation possibly accompanied by some denaturation.*

Wu and Yen (8) found that when egg albumin was denatured by alkali a decrease of alkalinity occurred. A similar, though smaller, decrease of acidity occurred when the denaturation was carried out in an acid solution. Pauli and collaborators (6) have previously found a decrease of alkalinity of an alkaline serum albumin solution on standing. This was attributed by Pauli (6) to an "alteration in state of the alkaline protein", but it was interpreted by Wu and Yen simply as the result of denaturation. Using a colorimetric method Wu and Yen (7) observed in the case of egg albumin a similar change in reaction as the result of heat denaturation uncomplicated by coagulation.

Harris (3) in the course of an investigation on a hitherto unknown sulphur group in the protein molecule found by formaldehyde titration no detectable change of amino-nitrogen content as the result of denaturation. Mastin and Schryver (5) found, however, an increase in the number of free acid groups without corresponding increase in the amino-groups when egg albumin was denatured by weak acids in the presence of salts.

In view of the confusion between denaturation and coagulation by some authors, the contradictory findings of others and the uncertainty of the colorimetric method for pH determination of protein solutions, a further study of the question with the electrometric method is desired.

EXPERIMENTAL

We have studied the changes in hydrogen ion concentration of protein solutions or suspensions (a) when denaturation occurs without the complication of coagulation, (b) when previously denatured albumin undergoes coagulation, and (c) when both denaturation and coagulation occur. In the first mentioned case the change is due to denaturation alone; in the second case, due to coagulation alone; and in the third to the combined effects of denaturation and coagulation.

It was found early in this study that albumin denatured by heating at different pH's had different acid- and base-binding powers, although heating for 5, 10 and 20 minutes gave practically the same results. To 30 cc of 0.64 per cent egg albumin 2.5 cc of N/10 HCl were added.

*See the preceding paper for definitions of these terms.

Four such tubes were prepared; one was kept as control while the other three were placed in boiling water bath. These tubes was removed, one at a time, from the bath at the end of 5, 10 and 20 minutes; cooled, the lost water replaced, and the pH determined. The pH of the control was 2.92, while those of the solutions heated for 5, 10 and 20 minutes were 3.09, 3.09 and 3.08 respectively. In a similar experiment with 1.1 per cent egg albumin using 1.9 cc of N/10 NaOH, the pH of unheated solution was 10.52, while those of the solution heated for 5, 10 and 20 minutes were 10.13, 10.12 and 10.12 respectively.

To determine the complete titration curve of each of the albumins denatured at different reactions would involve a large amount of unnecessary labor. It is sufficient for the present purpose to determine the acid-binding power at some convenient pH on the acid side of the isoelectric point of the protein and the base-binding power at a similar point of the alkaline side. A still simpler procedure is to determine the pH of the solution containing a particular amount of acid or alkali.

In most of the experiments the albumin solution were heated with different amounts of acid or alkali, but before measuring the pH the total acid or alkali contents of all solutions were made the same. The pH's of these solutions were compared with that of a single solution of natural albumin containing the same amount of acid or alkali as the denatured albumin solutions. If denaturation caused no change in acid- or base-binding power the final reaction of the heated solutions should be the same as that of the unheated control. If a change occurred, a difference would be found, and the amount of this difference, other things being equal, would depend on the pH of the control chosen for reference. It decreases as the "reference point" is moved away from the isoelectric point. The amount of acid or alkali used in the control solutions was so chosen that the denatured albumin showed no tendency to flocculate and yet not too acid or too alkaline to obscure the change in reaction.

The albumin solutions were prepared from dialyzed crystalline egg albumin. The reaction of the soultion was close to, but probably not always at, the isoelectric point of egg albumin. This condition was, however, not essential, since we were not interested in the absolute values, but rather in the changes, of the acid- and base-binding powers. The concentration of the albumin solutions used was 0.5 to 1.0 per cent.

The pH of the solutions was determined electrometrically using the quinhydrone electrode as far as possible. When this could not be used the hydrogen electrode was resorted to.

Effect of denaturation of egg albumin without coagulation.—Different amounts of N/10 HCl and N/10 NaOH were added to 25 cc portions of egg albumin solution contained in 25 × 200 mm test tubes. The minimum amount of acid or alkali used was sufficient to prevent coagulation. After mixing, the tubes were weighed to decigram and plunged simultaneously into a boiling water bath. The temperature of the bath dropped to 90° which was maintained for 10 minutes. At the end of this time the tubes were removed and cooled in running water. They were weighed and the loss of water replaced. The required amounts of acid or alkali were added to the tubes to equalize the total acid or alkali content. The volume of the solution was equalized by adding H_2O or appropriate mixtures of HCl and NaOH. After mixing, the pH of the solutions were measured and compared with the unheated control. The results of two experiments are shown in tables 1–2 and figs. 1–2. The point of reference was in table 1 on the acid side of the isoelectric point, and in table 2 on the alkaline side.

It will be noted that there is an increase in pH of the solution as the result of heat denaturation when the final reaction is measured on the acid side of the isoelectric point, and a decrease of pH when measured on the alkaline side.

The amount of change in pH increases as the reaction at which the albumin is denatured is further removed from the isoelectric point of this protein (pH 4.8). If the albumin were heated at this reaction it would have been coagulated and the effect of coagulation would be superimposed on that of denaturation. By extrapolation of the curves (dotted lines in figs. 1–2) it can be shown, however, that if albumin could be denatured at the isoelectric point without coagulation, there would be no change in acid- or base-binding power. A more plausible interpretation of this fact is that the albumin is not denatured at the isoelectric point under the conditions of our experiment.

TABLE 1.

Effect of denaturation on acid-binding power of egg albumin

25 cc 0.73 per cent egg albumin+graded amounts of N/10 HCl or N/10 NaOH.

	Before heating		After heating			
No.	N/10 HCl or N/10 NaOH added	pH of solution	N/10 HCl or N/10 NaOH added	N/20 NaCl added	pH of solution	pH difference from control
	cc		*cc*	*cc*		
1	2.0 HCl	2.53	0.0 HCl	4.0	2.68	0.12
2	1.5	2.71	0.5	4.0	2.64	0.08
3	1.0	3.03	1.0	4.0	2.61	0.05
4	0.8	3.19	1.2	4.0	2.59	0.03
5	0.6	3.41	1.4	4.0	2.59	0.03
6	0.4	3.71	1.6	4.0	2.58	0.02
7	0.6 NaOH	9.46	2.6	2.8	2.61	0.05
8	0.7	10.13	2.7	2.6	2.62	0.06
9	0.9	10.70	2.9	2.2	2.63	0.07
10	1.2	11.06	3.2	1.6	2.64	0.08
11	1.5	11.28	3.5	1.0	2.66	0.10
12	2.0	11.47	4.0	0.0	2.67	0.11
Unheated control			2.0	4.0	2.56	

TABLE 2.

Effect of denaturation on base-binding power of egg albumin

25 cc 0.7 per cent egg albumin+graded amounts of N/10 HCl or N/10 NaOH.

	Before heating		After heating			
No.	N/10 HCl or N/10 NaOH added	pH of solution	N/10 HCl or N/10 NaOH added	N/20 NaCl added	pH of solution	pH difference from control
	cc		*cc*	*cc*		
1	2.0 HCl	2.40	2.4 NaOH	0.6	9.63	−0.42
2	1.7	2.47	2.1	1.2	9.57	−0.48
3	1.3	2.65	1.7	2.0	9.43	−0.65
4	1.0	2.80	1.4	2.6	9.57	−0.48
5	0.8	2.97	1.2	3.0	9.68	−0.37
6	0.5	3.30	0.9	3.6	9.77	−0.28
7	0.16 NaOH	8.16	0.24	4.6	9.84	−0.21
8	0.26	8.42	0.14	4.6	9.62	−0.43
9	0.4	9.30	0.00	4.6	9.53	−0.52
10	0.6	9.88	0.20 HCl	4.2	9.09	−0.96
11	0.8	10.46	0.40	3.8	9.01	−1.04
12	1.2	10.90	0.80	3.0	8.34	−1.71
Unheated control	0.4			4.6	10.05	

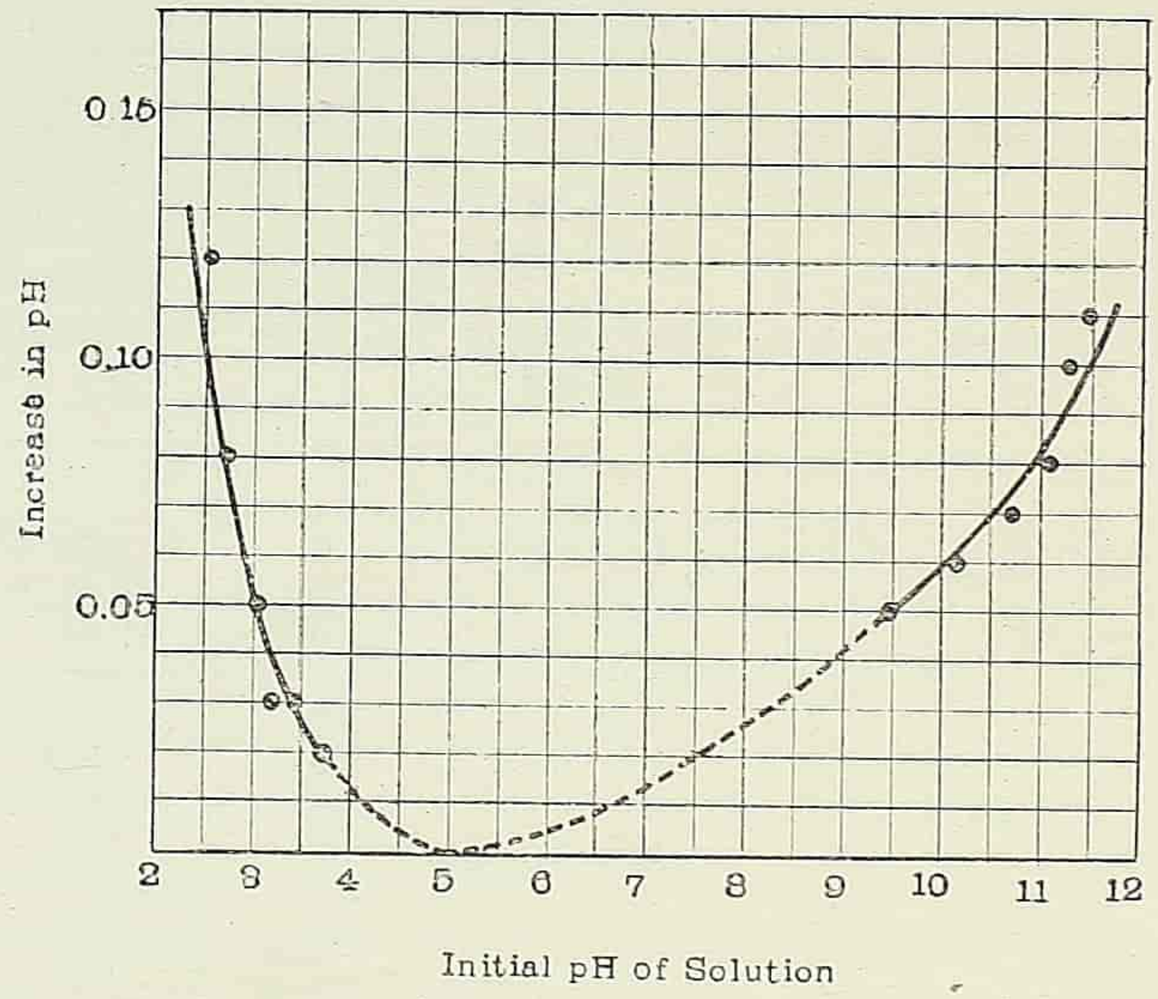

Fig. 1. Change in acid-binding power of egg albumin on denaturation.

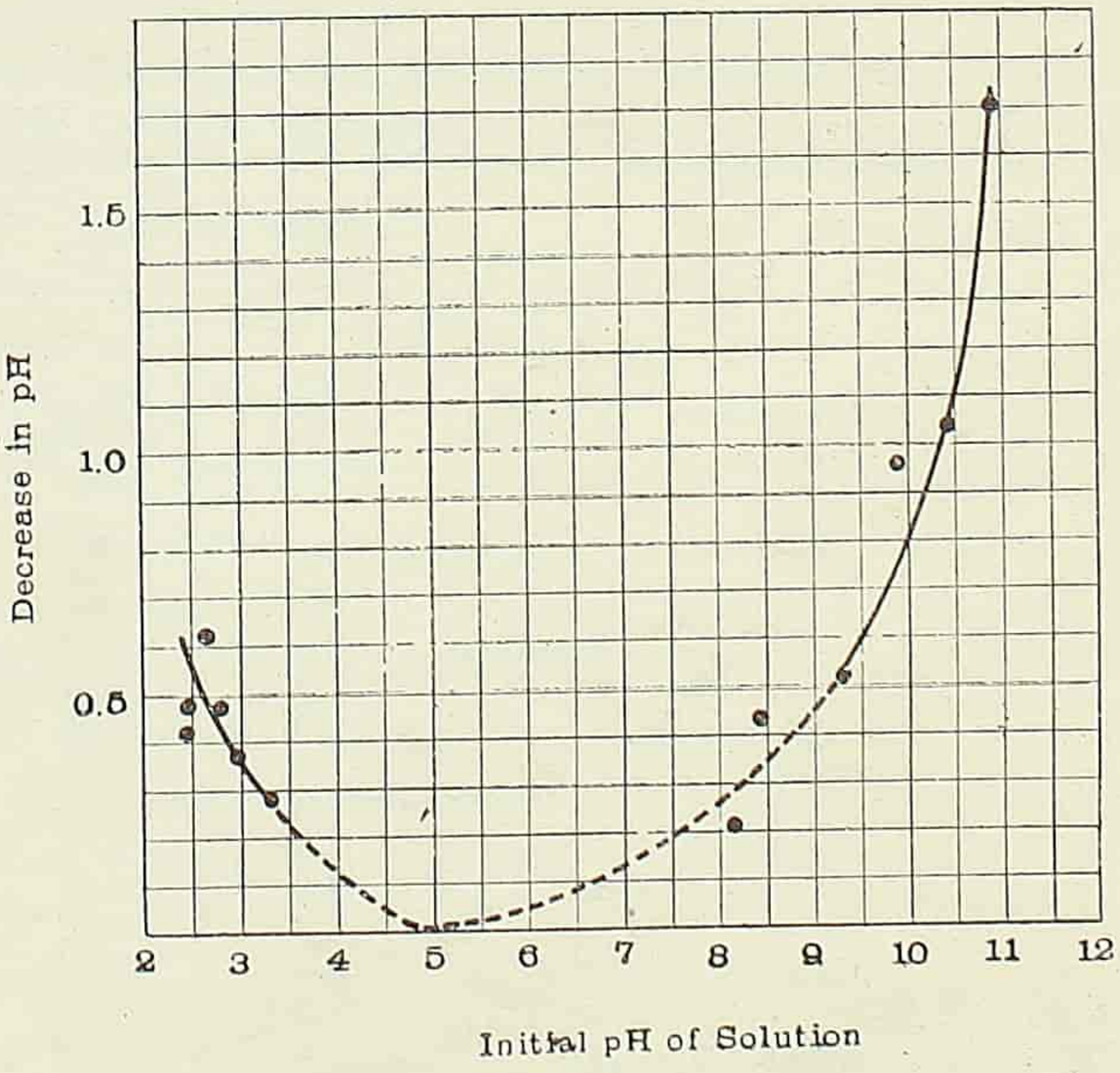

Fig. 2. Change in base-binding power of egg albumin on denaturation.

Effect of coagulation of egg albumin.—Some egg albumin was denatured by heating in acid. After cooling, the albumin was precipitated by neutralization. It was centrifuged and purified once by dissolving in dilute alkali and subsequent reprecipitation by neutralization. The denatured albumin was again dissolved in dilute alkali, giving an approximately one per cent solution. Equal portions of the solution were measured into test tubes, and different amounts of acid were added to give pH values from 4 to 6. These tubes were weighed and heated as described above. After heating, the total acid or alkali content of the tubes was equalized before measuring the pH. The results of two experiments are shown in tables 3–4 and fig. 3–4. The point of reference was in table 3 on the acid side of the isoelectric point and in table 4 on the alkaline side.

It will be noted that the coagulated solutions are more acid in acid solution and more alkaline in alkaline solution.

TABLE 3.

Effect of coagulation on acid-binding power of denatured (and flocculated) egg albumin

20 cc of 1 per cent denatured albumin in dilute alkali+graded amounts of N/10 HCl.

No.	Before heating	After heating		
	N/10 HCl added	N/10 HCl added	pH of suspension	pH difference from control
	cc	*cc*		
1	0.2	0.4	4.04	−0.10
2	0.3	0.3	3.92	−0.22
3	0.35	0.25	3.80	−0.34
4	0.4	0.2	3.81	−0.33
5	0.45	0.15	3.92	−0.22
6	0.5	0.1	4.02	−0.12
7	0.6	0.0	4.14	−0.00
Unheated control	0.6		4.14	

TABLE 4.

Effect of coagulation on base-binding power of denatured (and flocculated) egg albumin

20 cc 1 per cent denatured albumin in dilute alkali+graded amounts of N/10 HCl.

No.	Before heating	After heating			
	N/10 HCl added	N/10 HCl or N/10 NaOH added	N/10 NaCl added	pH of suspension	pH difference from control
	cc	*cc*	*cc*		
1	0.15	0.03	0.82	6.10	0.00
2	0.2	0.02 NaOH	0.78	6.19	0.09
3	0.25	0.07	0.68	6.32	0.22
4	0.3	0.12	0.58	6.32	0.22
5	0.35	0.17	0.48	6.26	0.16
6	0.40	0.22	0.38	6.23	0.13
Unheated control	0.18		0.82	6.10	

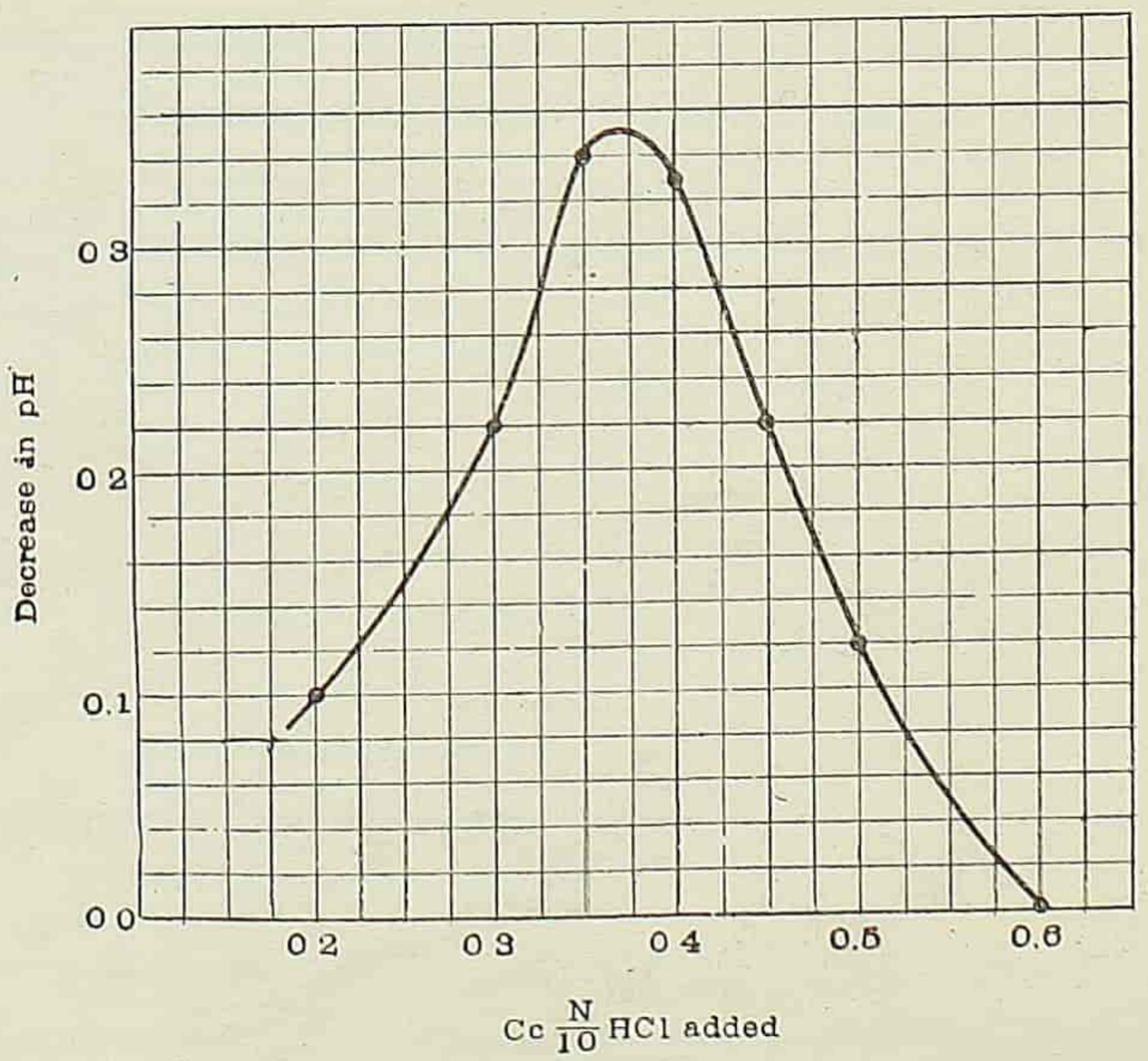

Fig. 3. Change in acid-binding power of denatured egg albumin on coagulation.

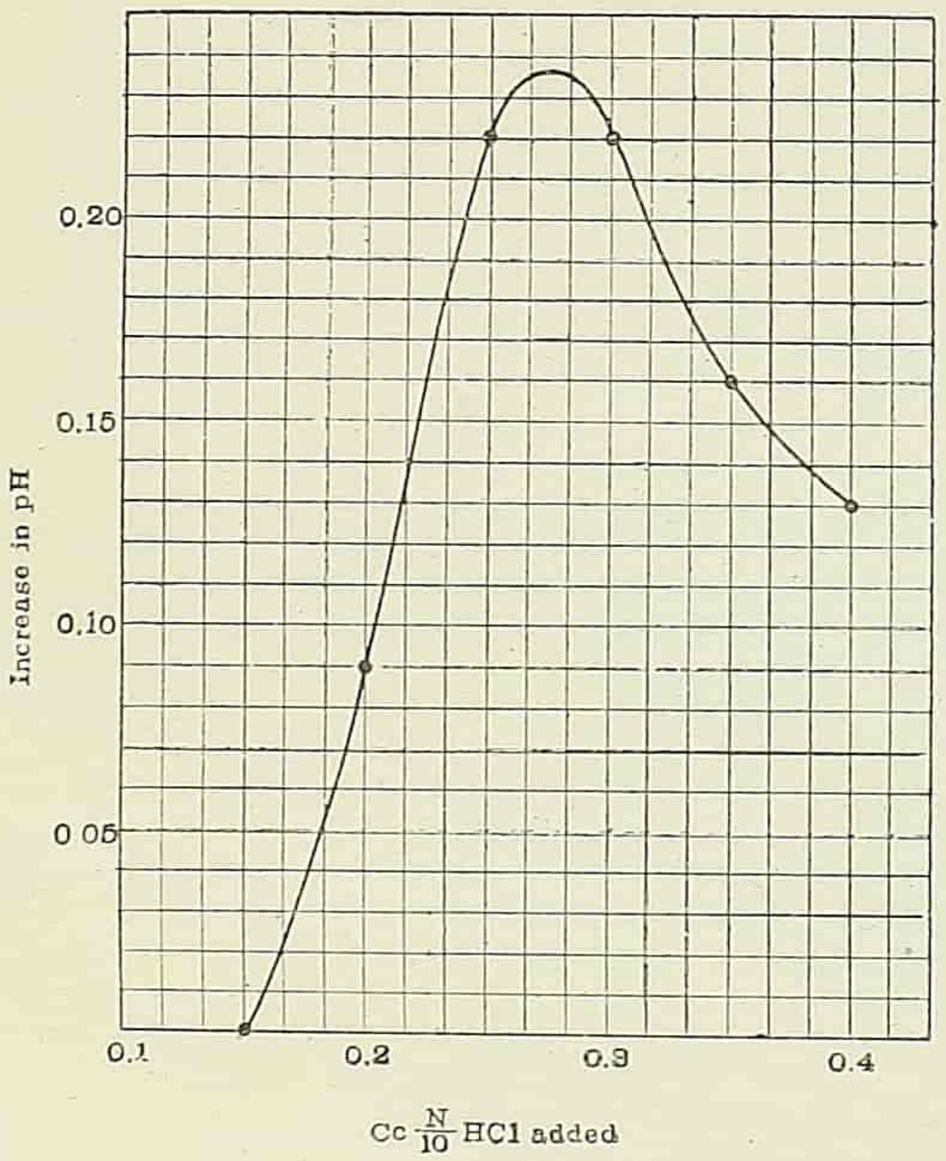

Fig. 4. Change in base-binding power of denatured egg albumin on coagulation.

Combind effect of denaturation and coagulation of egg albumin.—The procedure in these experiments was the same as that described above, except that in some solutions the amount of acid or alkali used was not enough to prevent coagulation. The results of two experiments are shown in tables 5–6 and figs. 5–6.

The results of these experiments confirm those in which denaturation and coagulation were studied separately. At and about the isoelectric point the effect of coagulation predominates over that of denaturation. In more acid or alkaline solutions, the reverse is the case. At two points, one on either side of the isoelectric point, the effects of denaturation and coagulation just balance each other and no change in pH was obesrved.

This is shown perhaps more clearly in another experiment in which the total acid or alkali contents of different solutions were not equalized before measuring the pH, but a control was prepared for each of the heated solutions. The results of this experiment are shown in table 7 and fig. 7. In areas I and IV the effect of denaturation predominates over that of coagulation, while in areas II and III the reverse is the case.

TABLE 5.

Effect of denaturation and coagulation on acid-binding power of egg albumin
25 cc 1 per cent egg albumin solutions + graded amounts of N/10 HCl or N/10 NaOH.

No.	Before heating		After heating			
	N/10 HCl or N/10 NaOH added	pH of solution	N/10 NaOH or N/10 HCl added	N/20 NaCl added	pH of solution or suspension	pH difference from control
	cc		*cc*	*cc*		
1	2.0 HCl	2.96	0.5 NaOH	5.0	3.80	0.38
2	1.8	3.08	0.3	5.4	3.62	0.20
3*	1.5	3.30	0.0	6.0	3.57	0.15
4*	1.2	3.61	0.3 HCl	6.0	3.53	0.11
5*	0.9	3.98	0.6	6.0	3.45	0.03
6*	0.7	4.29	0.8	6.0	3.29	−0.13
7*	0.5	4.66	1.0	6.0	3.25	−0.17
8*	0.3	5.10	1.2	6.0	3.28	−0.14
9*	0.2	5.31	1.3	6.0	3.26	−0.16
10*	0.1	5.58	1.4	6.0	3.29	−0.13
11*	0.0	5.90	1.5	6.0	3.35	−0.07
12*	0.1 NaOH	6.20	1.6	5.8	3.44	0.02
13*	0.2	6.62	1.7	5.6	3.49	0.07
14*	0.4	7.95	1.9	5.2	3.53	0.11
15	0.6	9.45	2.1	4.8	3.55	0.13
16	0.8	9.87	2.3	4.4	3.56	0.14
17	1.0	10.36	2.5	4.0	3.57	0.15
18	1.2	10.51	2.7	3.6	3.59	0.17
19	1.5	10.82	3.0	3.0	3.59	0.17
20	1.8	10.89	3.3	2.4	3.61	0.19
21	2.3	11.17	3.8	1.4	3.60	0.18
22	3.0	11.27	4.5	0.0	3.55	0.13
Unheated control			1.5	6.0	3.42	

*Solutions show turbidity or precipitate after heating.

TABLE 6.

Effect of denaturation and coagulation on base-binding power of egg albumin

25 cc 0.65 per cent egg albumin solution+graded amounts of N/10 HCl or N/10 NaOH.

No.	Before heating		After heating			
	N/10 HCl or N/10 NaOH added	pH of solution	N/10 NaOH added	N/20 NaCl added	pH of solution or suspension	pH difference from control
	cc		*cc*	*cc*		
1	1.8 HCl	2.59	2.6	0.6	9.97	−0.29
2	1.5	2.72	2.3	1.2	10.09	−0.17
3	1.2	2.91	2.0	1.8	10.14	−0.12
4	1.0	3.11	1.8	2.2	10.24	−0.02
5	0.8	3.25	1.6	2.6	10.22	−0.04
6	0.6	3.53	1.4	3.0	10.25	−0.01
7*	0.4	4.02	1.2	3.4	10.36	0.10
8*	0.3	4.24	1.1	3.6	10.60	0.24
9*	0.2	4.59	1.0	3.8	10.39	0.13
10*	0.1	4.90	0.9	4.0	10.36	0.10
11*	0.0	5.28	0.8	4.2	10.41	0.15
12*	0.1 NaOH	5.71	0.7	4.2	10.35	0.09
13*	0.15	5.98	0.65	4.2	10.25	−0.01
14*	0.2	6.21	0.6	4 2	10.29	0.03
15*	0.3	6.94	0.5	4.2	10.13	−0.13
16*	0.4	7.95	0.4	4.2	10.06	−0.20
17*	0.5	9.10	0.3	4.2	9.87	−0.39
18	0.6	9.32	0.2	4.2	9.86	−0.40
19	0.8	9.87	0.0	4.2	9.66	−0.60
Unheated control			0.8	4.2	10.26	

*Solutions show turbidity or precipitate after heating.

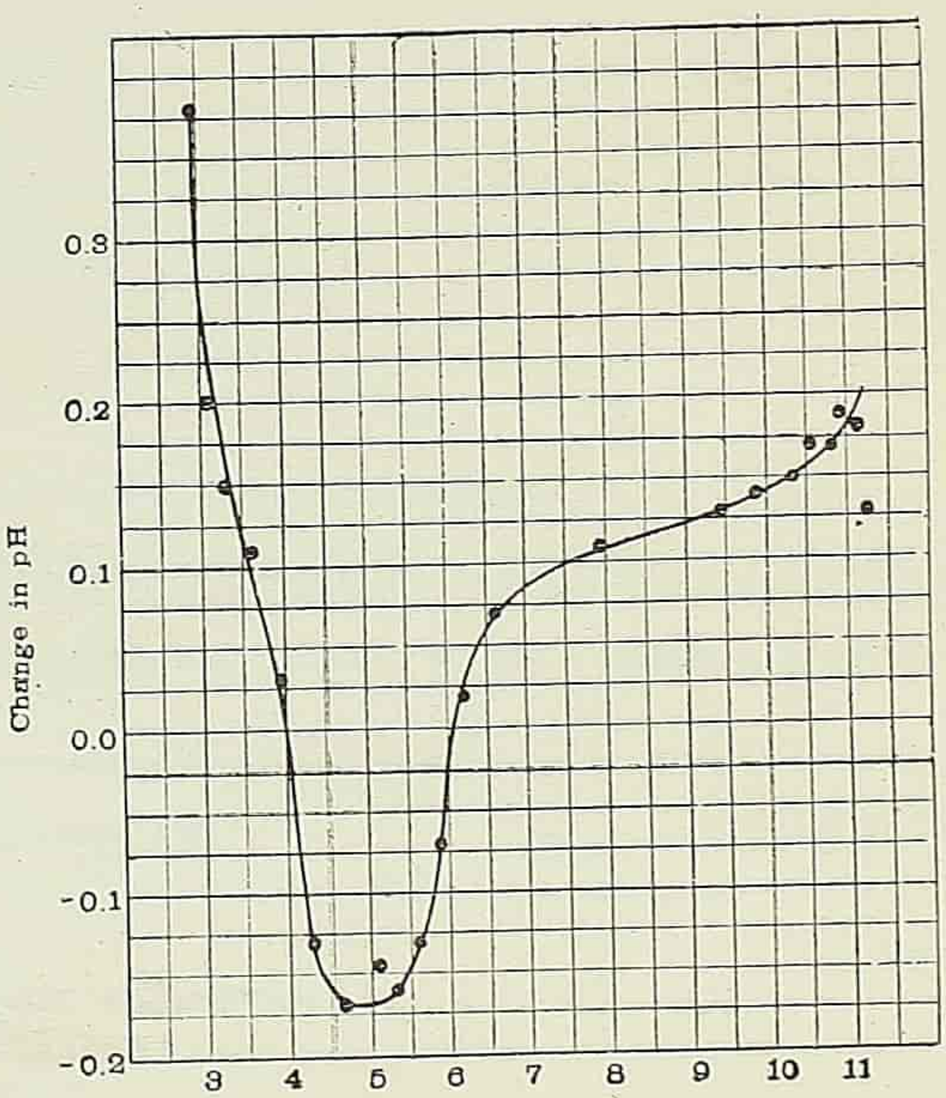

Fig. 5. Change in acid-binding power of egg albumin on denaturation and coagulation.

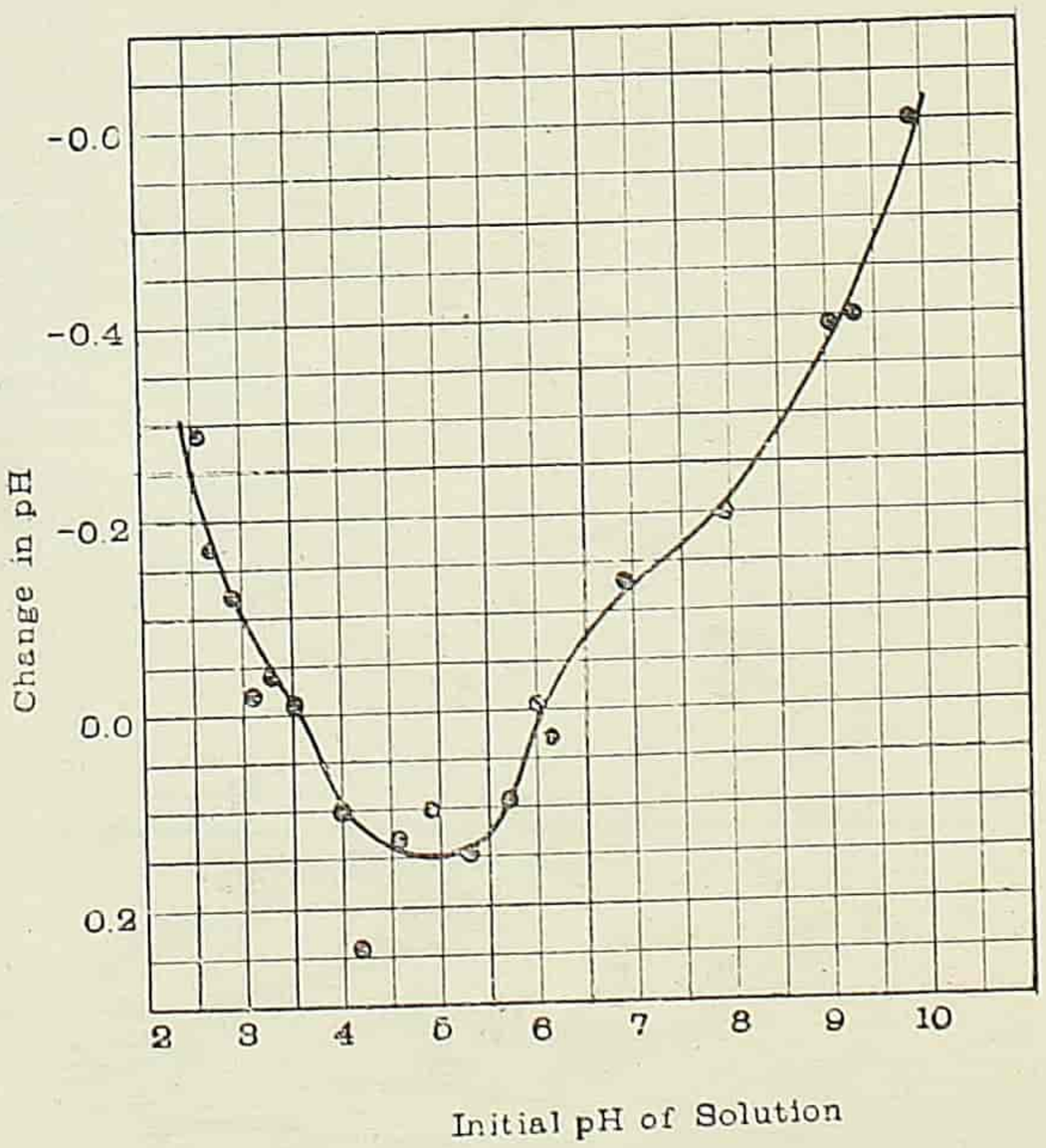

Fig. 6. Change in base-binding power of egg albumin on denaturation and coagulation.

TABLE 7.

Change of pH of egg albumin solution on denaturation and coagulation

30 cc ½ per cent egg albumin solution+graded amounts of N/10 HCl or N/10 NaOH.

No.	N/10 HCl or N/10 NaOH added	Water added	pH of unheated controls	pH of heated solution or suspension	pH difference
	cc	*cc*			
1	1.0 HCl	0.0	3.05	3.25	0.20
2	0.5	0.5	3.66	3.76	0.10
3	0.35	0.65	4.02	4.03	0.01
4*	0.2	0.8	4.45	4.40	−0.05
5*	0.1	0.9	4.93	5.67	0.71
6*	0.0	1.0	5.39	6.45	1.06
7*	0.05 NaOH	0.95	5.69	6.66	0.97
8*	0.15	0.85	6.27	6.96	0.69
9*	0.2	0.8	6.50	7.09	0.59
10*	0.3	0.7	7.10	7.46	0.36
11	0.4	0.6	7.52	7.50	−0.02
12	0.5	0.5	7.95	7.67	−0.28

*Solutions show turbidity or precipitate after heating.

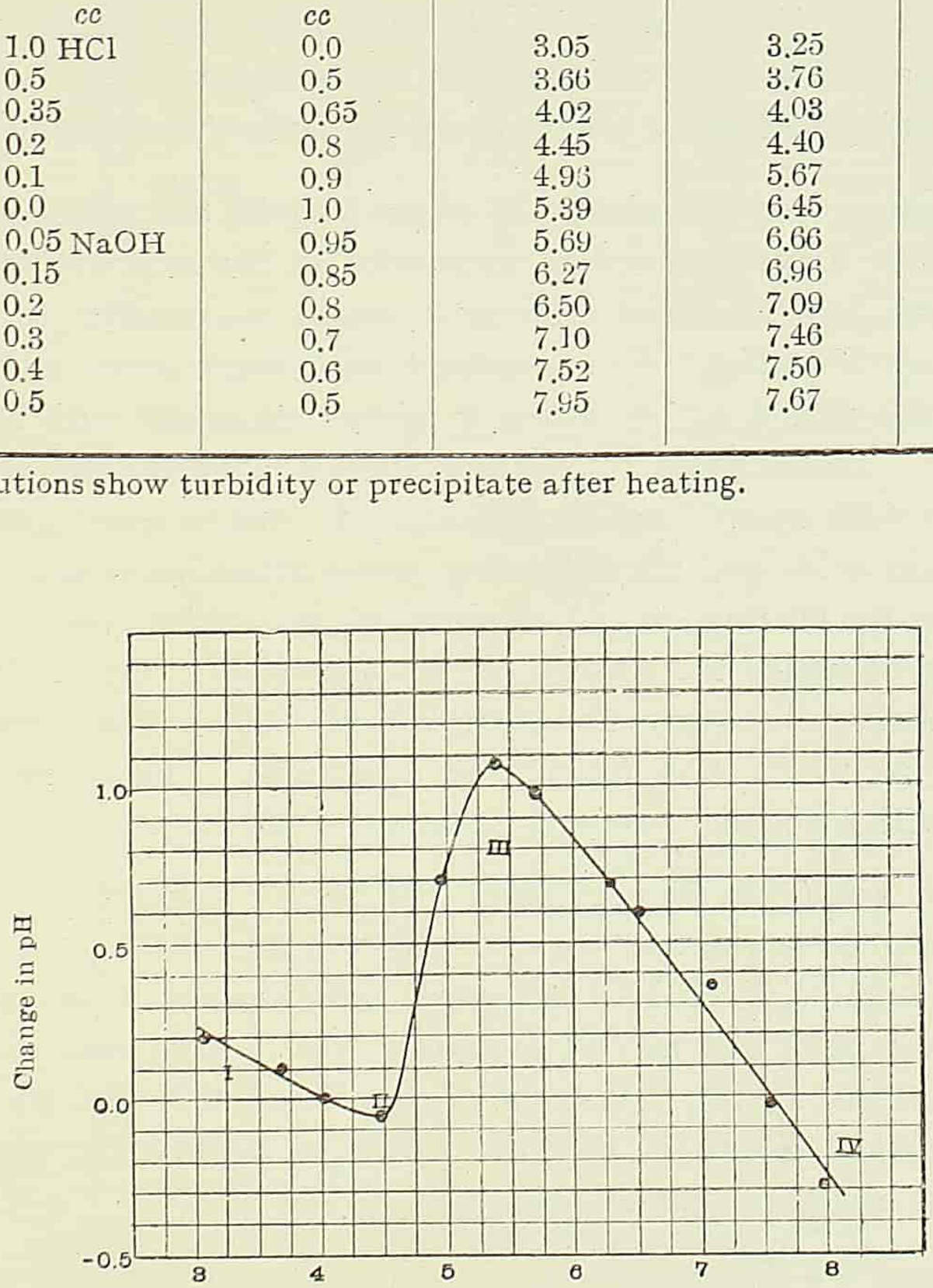

Fig. 7. Change in pH of egg albumin solution on denaturation and coagulation.

Denaturation of egg albumin by acid.—We have studied also the effect of denaturation of egg albumin by acid in the cold. Some 1 per cent egg albumin solution was mixed with an equal volume of N/10 HCl and allowed to stand over night. To 20 cc portions of the mixture were added 9.2, 9.0, 8.6, 8.2 and 7.5 cc of N/10 NaOH. The pH's of the resulting solutions were 3.63, 3.27, 2.82, 2.57 and 2.33 respectively. The pH's of the controls, prepared by mixing first the appropriate amounts of HCl and NaOH and then adding the albumin just before the pH determination, were 3.46, 3.17, 2.77, 2.54 and 2.32. These solutions of denatured albumin are thus less acid than those of the natural albumin. The decrease in alkalinity attending denaturation of albumin by alkali in the cold found in our previous study (8) was so large that a confirmation by electrometric methods is not necessary.

Denaturation and coagulation of serum globulin and albumin.—The results of these experiments were in general of the same kind as those obtained with egg albumin, although certain peculiarity of serum albumin is worth noting. An experiment with dog's serum globulin is shown in table 8 and fig. 8, which is quite comparable with fig. 5 for egg albumin. Tables 9 and 10 and figs. 9 and 10 show the results of two experiments with sheep's serum albumin. It will be noted that while the change in acid- and alkali-binding power attending coagulation or denaturation by heating on the alkaline side is qualitatively similar to that of egg albumin, the change on the acid side is quite different. Within a considerable range the change in pH is the same, irrespective of the pH at which the albumin is denatured. This behavior is associated with a peculiar property of serum albumin.

Wu and Yen (7) found previously that horse's serum albumin could not be denatured by mixing a one per cent solution of the protein with an equal volume of N/10 HCl, although denaturation did occur if the acid used was only half as concentrated. This is true even when the solution was heated (8). We have now found that sheep's serum albumin behaves similarly, but the range of acidity in which no denaturation occurs is wider.

This absence of denaturation is, however, only apparent and not real. When an acid solution of sheep's serum albumin heated for 10 minutes at 90°C is cooled and neutralized, the solution remains clear. If, however, anhydrous sodium sulphate is added to make a concentration of 20 per cent the albumin is partly precipitated. An unheated control under

TABLE 8.

Effect of denaturation and coagulation on acid-binding power of serum globulin

20 cc 0.8 per cent serum globulin solution+graded amounts of N/10 HCl or N/10 NaOH.

No.	Before heating		After heating			
	N/10 HCl or N/10 NaOH added	pH of solution	N/10 HCl or N/10 NaOH added	N/20 NaCl added	pH of solution or suspension	pH difference from control
	cc		*cc*	*cc*		
1	1.2 HCl	3.0	0.7 NaOH	2.1	4.12	0.19
2	0.9		0.4	2.7	4.14	0.21
3*	0.6		0.1	3.3	4.10	0.17
4*	0.4		0.1 HCl	3.5	4.06	0.13
5*	0.2		0.3	3.5	3.99	0.06
6*	0.0	5.2	0.5	3.5	3.83	−0.10
7*	0.2 NaOH		0.7	3.1	4.02	0.09
8	0.4		0.9	2.7	4.13	0.20
9	0.6		1.1	2.3	4.17	0.24
10	0.9		1.4	1.7	4.24	0.31
11	1.5	10.5	2.0	0.5	4.42	0.49
Unheated control			0.5	3.5	3.93	

*Solutions show turbidity or precipitate after heating.

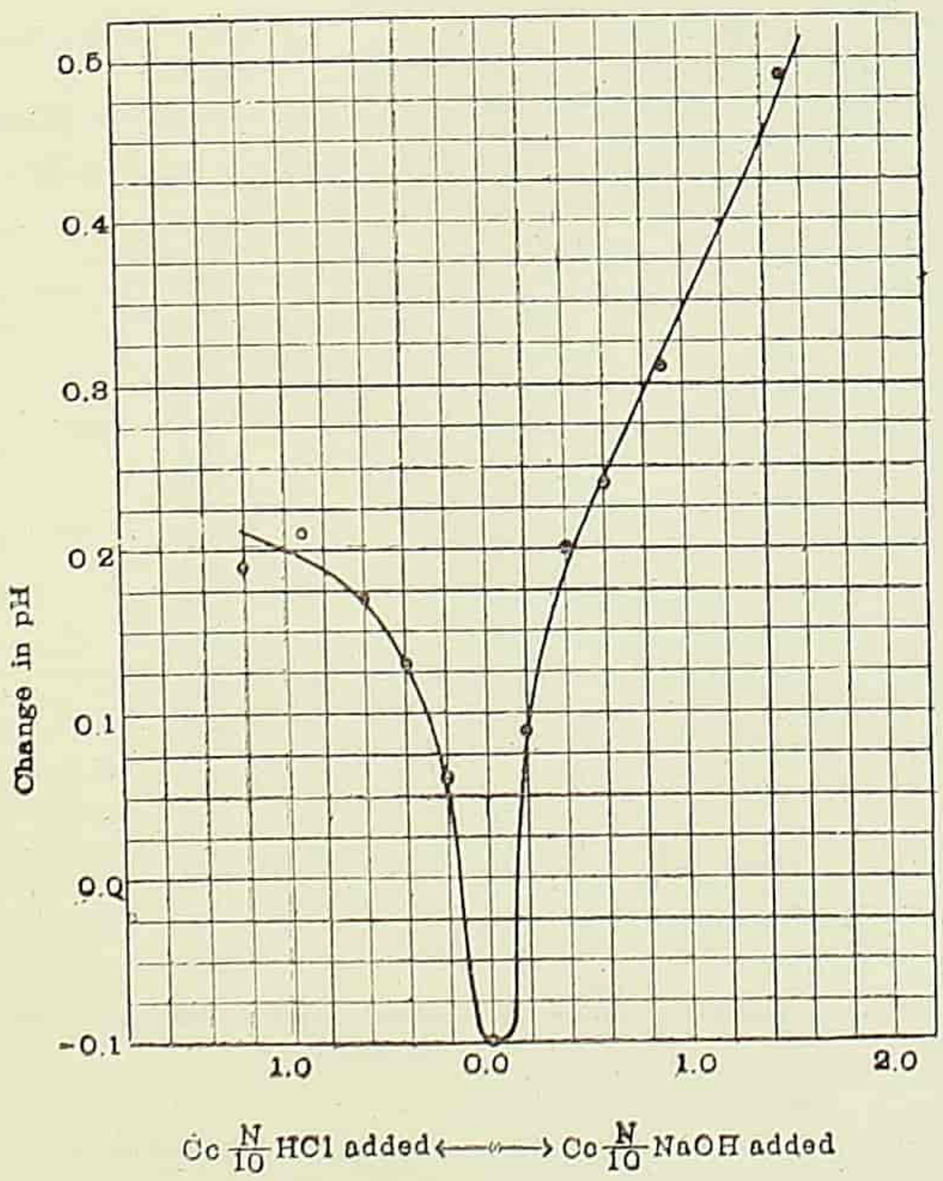

Fig. 8. Change in acid-binding power of serum globulin on denaturation and coagulation.

the same conditions remains clear.** This shows that the albumin has undergone a change,—corresponding to the change is acid- and base-binding power expressed by the horizontal portion of the curves in figs. 9 and 10.

The effect of acid on sheep's serum albumin in the cold is very slow. A $\frac{1}{2}$ per cent solution was mixed with an equal volume of N/10 HCl. After 24 hours the acid-binding power was measured at different reactions as described above for egg albumin and no change was detected. Addition of sodium sulphate to make a concentration of 20 per cent, however, produced some turbidity. Probably denaturation will occur on longer standing.

TABLE 9.

Effect of denaturation and coagulation on acid-binding power of serum albumin

20 cc of 0.65 per cent serum albumin solution+graded amounts of N/10 HCl or N/10 NaOH.

No.	Before heating		After heating			
	N/10 HCl or N/10 NaOH added	pH of solution	N/10 HCl or N/10 NaOH added	N/20 NaCl added	pH of solution or suspension	pH difference from control
	cc		*cc*	*cc*		
1	1.8 HCl	2.77	1.3 NaOH	0.9	4.12	0.07
2	1.5		1.0	1.5	4.14	0.09
3	1.2		0.7	2.1	4.13	0.08
4	0.9		0.4	2.7	4.14	0.09
5	0.7		0.2	3.1	4.19	0.14
6*	0.5		0.0	3.5	4.14	0.09
7*	0.3		0.2 HCl	3.5	4.10	0.05
8*	0.2		0.3	3.5	3.99	−0.06
9*	0.1	5.37	0.4	3.5	3.88	−0.17
10*	0.0	5.82	0.5	3.5	3.88	−0.17
11*	0.05 NaOH		0.55	3.4	3.93	−0.12
12*	0.1		0.6	3.3	4.09	0.04
13	0.2		0.7	3.1	4.17	0.12
14	0.3		0.8	2.9	4.18	0.13
15	0.5	8.30	1.0	2.5	4.26	0.21
Unheated control			0.5	3.5	4.05	

*Solutions show turbidity or precipitate after heating.

**Wu and Yen used sodium sulphate to promote flocculation of denatured albumins in their study (7), but through an oversight this fact was not mentioned.

TABLE 10.

Effect of denaturation and coagulation on base-binding power of serum albumin

20 cc of 0.55 per cent serum albumin solution+graded amounts of N/10 HCl or N/10 NaOH.

No.	Before heating		After heating			
	N/10 HCl or N/10 NaOH added	pH of solution	N/10 HCl or N/10 NaOH added	N/20 NaCl added	pH of solution or suspension	pH difference from control
	cc		*cc*	*cc*		
1	2.0 HCl	2.38	2.4 NaOH	0.6	10.22	0.00
2	1.5	2.61	1.9	1.6	10.15	−0.07
3	1.0		1.4	2.6	10.15	−0.07
4	0.8		1.2	3.0	10.16	−0.06
5	0.5		0.9	3.6	10.16	−0.06
6	0.3		0.7	4.0	10.18	−0.04
7*	0.2		0.6	4.2	10.31	0.09
8*	0.1	5.64	0.5	4.4	10.36	0.14
9*	0.0		0.4	4.6	10.24	0.02
10*	0.1 NaOH		0.3	4.6	10.12	−0.10
11	0.2		0.2	4.6	10.01	−0.21
12	0.4	10.22	0.0	4.6	9.77	−0.45
13	0.6		0.2 HCl	4.2	9.34	−0.88
14	0.8	10.94	0.4	3.8	8.97	−1.25
Unheated control	0.4			4.6	10.22	

*Solutions show turbidity or precipitate after heating.

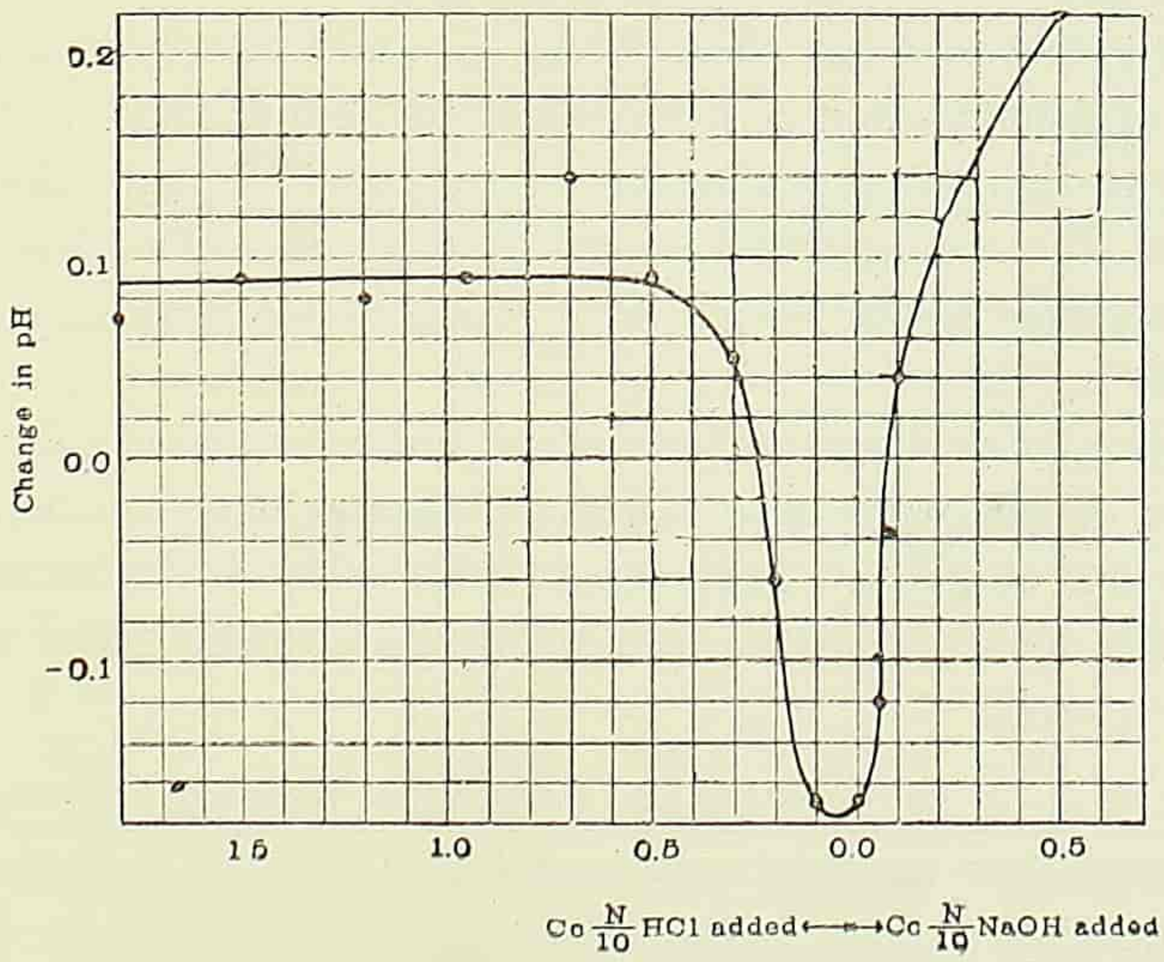

Fig. 9. Change in acid-binding power of serum albumin on denaturation and coagulation.

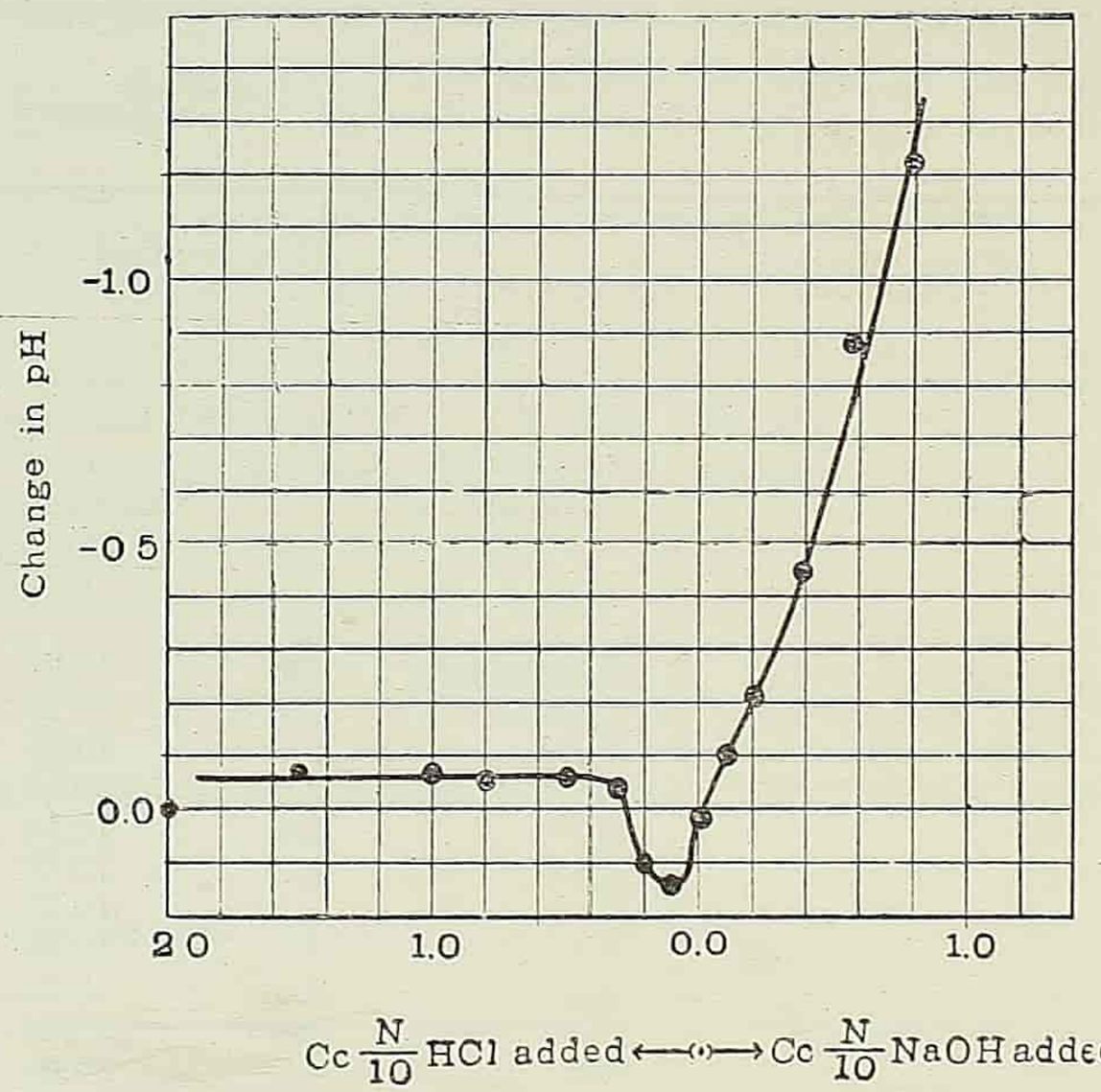

Fig. 10. Change in base-binding power of serum albumin on denaturation and coagulation.

Denaturation and coagulation of hemoglobin.—Table 11 and fig. 11 show the effect of heat denaturation and coagulation of oxyhemoglobin. The similarity between egg albumin (fig. 5) and hemoglobin is apparent. The effect of denaturation of hemoglobin by acid or alkali in the cold is also similar to that on egg albumin, the only difference being that the former is much more sensitive, especially towards acids. Hence in the more acid solution the difference in reaction between the denatured and the "control" could not be detected as the hemoglobin in the "control" also underwent denaturation before the measurement could be made. The change of pH with time of an acid hemoglobin solution with an initial reaction of pH 3.8 is shown in table 12. At an initial reaction of pH 3.0 the denaturation was practically instantaneous and no control could be prepared.

Lewis' failure to detect any difference between denatured and natural hemoglobin is to us quite puzzling. Most of the points which he determined lay outside of the pH range 5–8, and in view of the ease with which hemoglobin is denatured by dilute acids and alkalies, the

hemoglobin in his controls might have been denatured by the added acid or alkali before the measurement of pH could be made. This is unquestionably the case in the more acid solutions.

TABLE 11.

Effect of denaturation and coagulation on acid-binding power of oxyhemoglobin

20 cc 0.85 per cent oxyhemoglobin solution+graded amounts of N/10 HCl or N/10 NaOH.

No.	Before heating		After heating			
	N/10 HCl or N/10 NaOH added	pH of solution	N/10 HCl or N/10 NaOH added	N/20 NaCl added	pH of solution or suspension	pH difference from control
	cc		*cc*	*cc*		
1	1.5 HCl	3.85	0.7 NaOH	1.6	5.32	0.69
2	1.2		0.4	2.2	5.19	0.56
3	0.8		0.0	3.0	5.12	0.49
4*	0.5		0.3 HCl	3.0	4.97	0.34
5*	0.3		0.5	3.0	4.82	0.19
6*	0.1	6.78	0.7	3.0	4.34	−0.29
7*	0.0		0.8	3.0	4.51	−0.12
8*	0.1 NaOH		0.9	2.8	4.39	−0.24
9*	0.3		1.1	2.4	4.92	0.29
10*	0.5		1.3	2.0	4.97	0.34
11	0.8		1.6	1.4	5.01	0.38
12	1.2		2.0	0.6	5.11	0.48
13	1.5	10.06	2.3	0.0	5.14	0.51
Unheated control			0.8	3.0	4.63	

*Solutions show turbidity or precipitate after heating.

TABLE 12.

Change of pH of acid methemoglobin solution with time

100 cc of 1 per cent methomoglobin solution+4.8 cc N/10 HCl.

Time after mixing	3.4 mi.	9 mi.	25.7 mi.	71.7 mi.	Next morning
pH	3.82	3.86	3.93	3.99	4.17

Coagulated hemoglobin could not be redissolved in such dilute acid and alkali solutions in the pH range 5 to 8, and the reaction of such a suspension is necessarily different from the control in which the natural

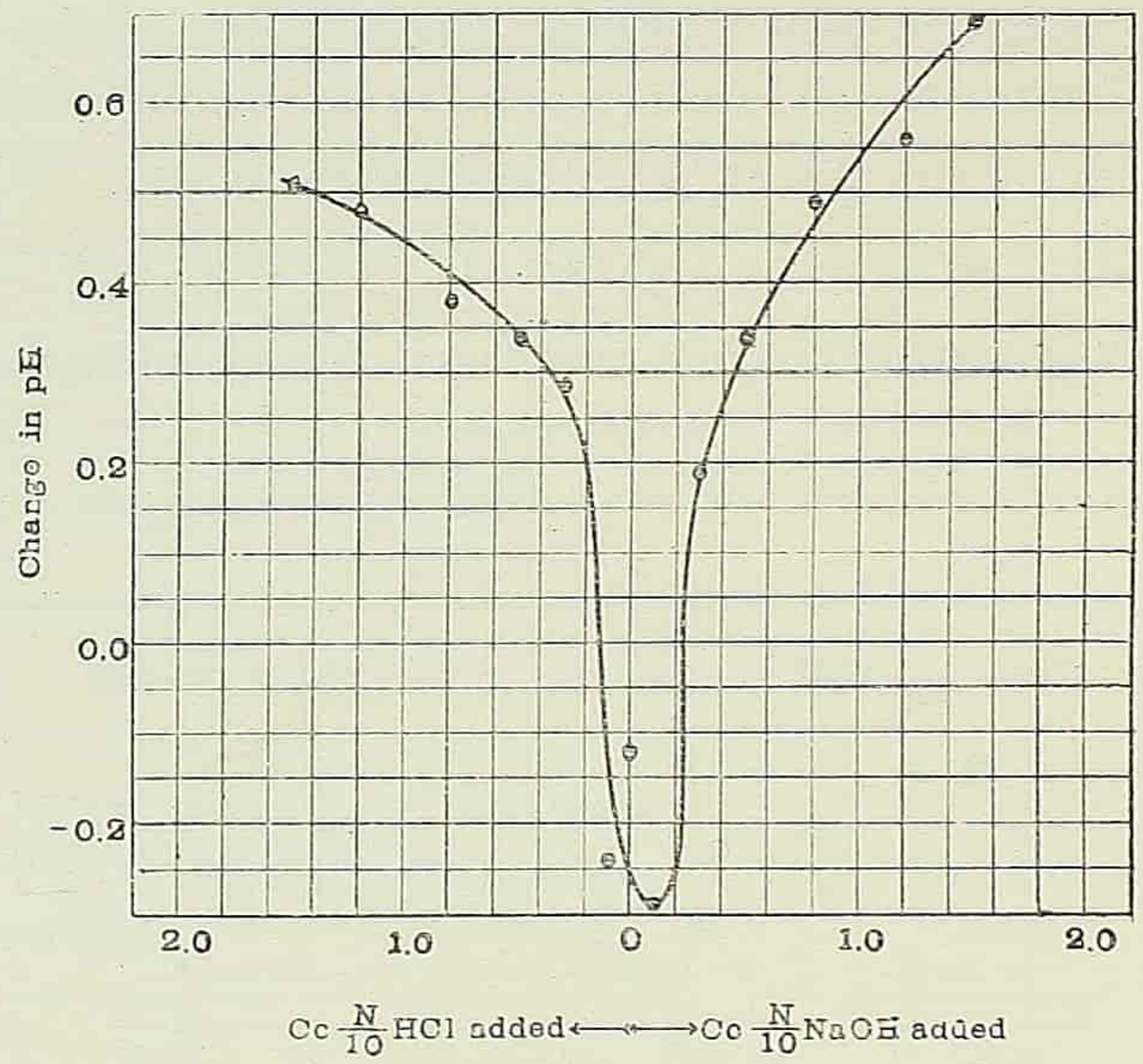

Fig. 11. Change in acid-binding power of oxyhemoglobin on denaturation and coagulation.

protein is in solution. Lewis made no mention of any difficulty encountered in bringing the coagulum into solution. We thought that if the coagulation occurred in the absence of any electrolyte the coagulum might be so fine and would redissolve in very dilute acids and alkalies. Accordingly we dialyzed some crystalline dog's oxyhemoglobin until free from salt, but the coagulum prepared from this was also insoluble. Of course there is the possibility that the stock hemoglobin solution which Lewis used was so acid or alkaline that no coagulation occurred in his experiment. In that case, it is probable that he was experimenting with hemoglobin which was already denatured.

DISCUSSION

That the solutions of the denatured protein are less acid in acid solution and less alkaline in alkaline solution than those of the corresponding natural protein can be interpreted as an increase of acid- and base-binding power of the protein on denaturation. The opposite phenomenon observed in coagulation, is, however, open to several interpretations. (A) The coagulated protein may have fewer acid- and base-binding groups due to some change in the protein molecule. (B) The coagulated protein, being less dispersed than the natural or flocculated protein, may require longer time to complete the combination

with acid or alkali than has been allowed in our experiments. (C) When the flocculated protein is coagulated, the adsorption of acid or alkali, if there is any at all, may be decreased. (D) When the coagulum is formed from the natural protein, the change from a homogenous solution to a suspension will cause unequal distribution of acid or alkali between the solution and in the suspended particles, the solution being more acid or alkaline according as the reaction of the original solution is on the acid or alkaline side of the isoelectric point. All these interpretations may be correct, but it is to be noted that while a change in the protein molecule can be the cause of coagulation, a change in the rate of combination, in the power adsorption or in the distribution of acids or alkalies are only the results of the physical change characteristic of coagulation, whatever be the cause of this phenomenon.

SUMMARY

Denaturation of protein is accompanied by a decrease of hydrogen ion concentration in acid solution or a hydroxyl ion concentration in alkaline solution. This may be interpreted as an increase of acid- and base-binding power, and supports the theory that the process underlying denaturation is one of degradation.

Coagulation has just the opposite effect. This may be explained as due to a condensation of the protein molecule with a decrease in the number of acid- and alkali-binding groups, but other explanations are also offered.

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蛋白質之變性作用

八. 變性作用及凝固作用對于蛋白質之酸類或鹼類化合能力之影響

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本篇目的: 爲研究變性作用之性質，究爲縮合作用乎，仰爲分解作用乎。如係後者則與酸類或鹼類之化合能力當因變性而增加，如係前者則反是。

研究方法: 以不同量之鹽酸或苛性鈉，加于同量之蛋白質溶液內，加熱使之起變性作用或凝固作用。冷後再加不同量之鹽酸或苛性鈉使各溶液所含之鹽酸或苛性鈉之量相等。然後用電量法測定其酸度或鹼度。另以本性蛋白質作比較之標準。

結果: 變性蛋白質與酸類或鹼類之化合能力比本性蛋白質大。凝固作用之影響則適相反。

結論: 上述蛋白質變性作用之結果可以加水分解或他種分解作用解釋之。凝固作用之結果則可有數種解釋，但以縮合作用爲最妥。

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THE RELATION OF THE MITOCHONDRIA-GOLGI COMPLEX TO SECRETION
III. PHYSIOLOGICAL IDENTIFICATION OF THE VITALLY STAINED MITOCHONDRIA-GOLGI MATERIAL

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In a previous paper (29) the technique of intravital staining with neutral red and Sudan III was given as a method for demonstrating the Golgi material. The aim of the present communication is to substantiate this claim by showing that the neutral red and Sudan III stained material corresponds to the osmiophile material obtained by the impregnation technique, in the resting and active gland, when simultaneous specimens are taken from the same tissue.

MATERIAL

The pancreas of rabbits and dogs was studied. Under urethane (for rabbit) or veronal (for dog) anaesthesia, the mixture of neutral red and Sudan III was injected and the pancreatic ducts cannulated. Small, thin pieces of pancreas were removed with scissors, partly for fresh examination and partly for osmium impregnation. It was found that removal of tissue in between ligatures for haemostasis would seriously alter the normal position of the Golgi-mitochondria material. Bleeding was fully checked after excision of the tissue sample. A sketch of the fresh preparation was recorded for future reference and comparison. The tissue had to be handled with great care in order to preserve the normal topography of the intracellular structures. Pilocarpine was given to excite activity, and pancreatic juice collected to control the degree of activity evoked. Tissues were removed periodically during the height and at the end of secretion. Similar observations were made on the salivary glands for the purpose of comparison.

OBSERVATION

The neutral red and Sudan III stained elements are located between the nucleus and the secretion granules. They consist of a number of droplets or rods with diameters larger than those of mitochondria and zymogen granules (figs. 33 and 34). The droplets are not uniform in size, the larger being situated below the secretion granules, while the small granules are usually found between the deeply placed zymogen granules. The droplets vary also in form. They may be somewhat elongated, rod-like, spherical or irregular.

It is probable that the red-stained droplets are able to coalesce, since a network is sometimes present. It may be mentioned that we have seen such a change to take place in the ciliated epithelium of the toad's oesophagus during ciliary activity (28). The changes in the pancreas are apparently too slow to be followed in this manner. Professor Cowdry has succeeded, however, in observing the complete genesis of the Golgi apparatus from the neutral red stained granules in Plasmodium praecox (13).

The droplets revealed by different neutral red methods, have been considered as vacuoles or granules within the Golgi area, but not as the Golgi elements themselves (3,34). The positive results of Ludford (22), Jasswoin (17), Zweibaum and Elkner (37), Chlopin (11), Arnold (2) etc., according to Bowen (9) "are all explicable on the basis of the known behaviour of this dye towards watery vacuoles". Neutral red, however, is also soluble in lipoid. Furthermore, if oleic acid or lecithin is added to a watery solution of neutral red, the fat will remove the stain from the watery solution—almost completely if the solution is dilute. In our judgment the neutral red-Sudan III droplets stained by our technique are at least as real as other cytoplasmic inclusions, for they can be extruded outside the cell body by the pressure of a heavy cover glass.

The droplets in the pancreatic cell as well as in the salivary glands change according to the functional state of the cell. In the loaded cell, they are very compact and chiefly located below the secretion granules. A few smaller red droplets may be scattered here and there throughout the zymogen granules (see figs. 17 and 33). When the secretion is being discharged, they become less compact and they are found more or less separate among the zymogen granules, their location being moved gradually toward the luminal end of the cell (figs. 5, 9, 13, 21, 25, 29, 34).

The nature of the changes exhibited by the red stained bodies during physiological activity lead us to suspect that they are closely associated with the Golgi material, while the fact that both neutral red and Sudan III are soluble in lipoid, and especially that lipoid will remove neutral red from water at once suggests that the Golgi material or some part of it is the substance coloured. This belief was further strengthened by the observation that both osmium and neutral red–Sudan III methods reveal the same changes, progressing at the same rate in the osmiophile and red material (Golgi lipoid) of one gland, whether pancreas or salivary (figs. 1–15 and 17–31). We have thus no hesitation in affirming that the neutral red-Sudan III method stains the Golgi material.

There is one point which must be emphasized. While in osmium preparations, the Golgi lipoid appears as a plexus or network, the neutral red-Sudan III stained preparation reveals chiefly droplets, a network being occasionally found. Whether or not any alteration of Golgi lipoid occurs after impregnation in the warm OsO_4 bath or whether the neutral red-Sudan III method stains only a part of the Golgi elements cannot be decided at present.

Mitochondria were again found in connection with the Golgi elements in the fresh material, the club-shaped mitochondria being not infrequently stained red at its enlarged end (fig. 34). This indicates, as we have pointed out before, that the Golgi lipoid is probably a product of mitochondrial dissociation. It should be added that the Golgi material (i.e. substances stainable by osmium, silver or neutral red-Sudan III method) is not only found at the ends but also in the body of mitochondria.

Lastly, we wish to draw attention to figs. 35–38. Concerning the fate of the Golgi material, it has been shown that, in the gastric glands, part is extruded (Ma, Lim and Liu, 25; Ma, 27). It has been abundantly demonstrated that Golgi material may be extruded by other glands, viz., mammary glands (Da Fano, 14; Beams, 4), sebaceous or oil glands (Bowen, 8), epithelia of oviduct (Brambell, 7). In the figures referred to it will be noted that as secretion progresses the Golgi material fragments and the fine particles are poured into the lumen along with a fine coagulate. We have found that osmiophile particles are present in the lumen of the ligatured pancreatic duct after stimulation of the gland, and that those particles behave like Golgi lipoid in being dissolved with difficulty by turpentine. They are perhaps more readily soluble

than the lipoid in the cell, suggesting that some change occurs on extrusion.

The present observations on pancreas and salivary glands show that the extrusion of a part of the Golgi lipoid is a fairly general phenomenon. It is interesting to note that neutral red passes into all the secretion, and colours them according to the reaction of the secretion fluid, e.g., red in gastric juice and urine, and yellow in saliva and pancreatic juice. In the living cell, however, neutral red is seen only in the Golgi region and perhaps, during active secretion, in the granular zone as well. It is invariably red in the Golgi region, but between the zymogen granules a yellow colour is sometimes seen. That is to say, the neutral red is not distributed elsewhere in the cell but is concentrated in the Golgi material although the dye is freely water soluble. This is to be expected, however, in view of the (selective?) affinity of lipoid substances for neutral red. When the material breaks down, the dye escapes into the secretion fluid and colours it. It would be interesting to know how much dye diffuses directly from blood to gland lumen, and how much is conveyed through the Golgi lipoid.

Evidence indicating that the remainder of the Golgi lipoid may be oxidized or resynthesized, has been obtained by Ni and Lim (33) and Ling, Liu and Lim (22) to whose papers the reader is referred.

DISCUSSION

Neutral red can stain various elements in the living cell, such as intracellular canaliculi in the oxyntic cells of the stomach and Langerhans' islands of the pancreas (5), granules in the kidney (1), granules in cultures of different tissues (21), (haemoglobinogenous) granules of blood cells (17), vacuoles (3,34) etc.

In identifying the neutral red-Sudan III stained material, we feel certain that its behaviour during rest and activity is identical with that of the Golgi lipoid revealed by the osmium technique. While it is probable that not everything which is stained by neutral red intravitally is Golgi material, it would be worth while to check the intravital neutral red stained granules of different tissues as reported by other workers with the standard osmium technique.

The name "Golgi apparatus" or "apparato reticolare interne" implies a network structure as usually obtained by silver or osmium methods. In the pancreas, such a structure was described by v. Bergen (6), Kolster (20), Cajal (10), Bowen (8), Saguchi (34), and others, and in the salivary gland by v. Bergen (6), Bowen (8), etc. Using

intravital staining with neutral red Chlopin (11) described a network, while Michaelis (30) observed granules chiefly. Our examination of the "structure" of Golgi material in living tissue leads us to the conclusion that the Golgi "apparatus" consists of a number of droplets which may at times coalesce, depending upon the condition at the interphase between Golgi material and surrounding medium. This change has been actually observed by us in vivo in the ciliated epithelium of the toad's mouth, and by Covell and Scott (12) and Dawson (15) in other material. The notion that the Golgi apparatus exists as a structural network (see Bowen, 9) or lamellae (Morelle, 31) can no longer be held, on the other hand the conception of it as a somewhat viscid fluid is more in accordance with its behaviour in the living cell.

Trypan blue is another vital stain with which Jasswoin (18) Nassonov (32) and Glasunow (16) have demonstrated the formation of a typical reticulum of Golgi material by the union of stained "granules". Recently Ludford in his paper on the staining of the Golgi apparatus in liver and kidney by trypan blue remarked that "It has been noticed occasionally in examining sections that part of the apparatus has reduced the osmic acid and appears black, while other parts seemed to be stained blue. Such an appearance is probably due to the dye (trypan blue) accumulating at the surface of the apparatus and opposing a barrier to the penetration of the osmic acid." Ludford believes that the trypan blue picture confirms Nassonov's theory that elaborated secretion material undergoes elective concentration into granules or droplets at the surface of the Golgi apparatus. Our view given in detail elsewhere (Ma, Lim and Liu, 25; Ma, 26) is that the Golgi material is derived by dissociation of the mitochondria, at the time when secretion material is elaborated. It may be pointed out that both Nassonov's and Ludford's observations were carried out on the liver and kidney, both organs whose cells are able to transfer substances from the blood across their cytoplasm to the luminal surface (or vice versa in the kidney) apparently unchanged. In this sense, Nassonov's interpretation of the trypan blue effect may be accepted, but it can hardly be generalized so as to apply to true secretory activity. Further, trypan blue does not stain the same material as neutral red intravitally (at least not in the pancreas and salivary gland), and is not soluble in fat or lipoid.

SUMMARY

A detailed study of the neutral red-Sudan III stained material of the pancreatic and the salivary cell has been made during rest and

during activity provoked by pilocarpine. Observations on identical material show that the behaviour of the neutral red-Sudan III stained material is similar to that of the osmiophile Golgi lipoid, thus establishing the identity of the two stained substances. Further Sudan III is only soluble in neutral red solution and not in water, while neutral red is lipoid soluble, and in a mixture of lipoid and water, the lipoid takes up most of the red. Since the Golgi material is the largest mass of lipoid in the cell, the presence of neutral red in the Golgi lipoid is accounted for.

Further evidence of the fate of some of the Golgi lipoid by discharge into the gland lumen is given.

We take pleasure in recording here our thanks to Dr. R.K.S. Lim for his suggestions.

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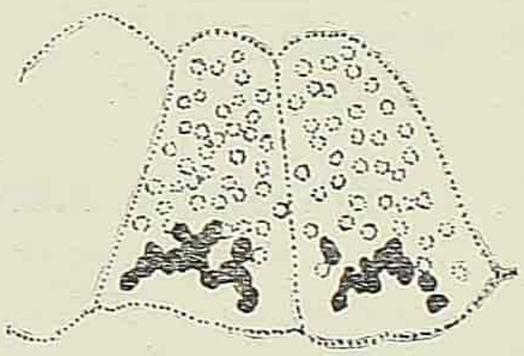
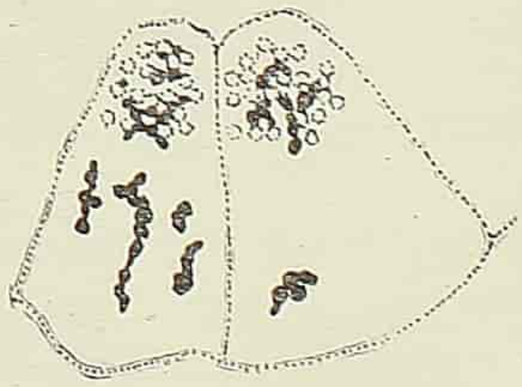
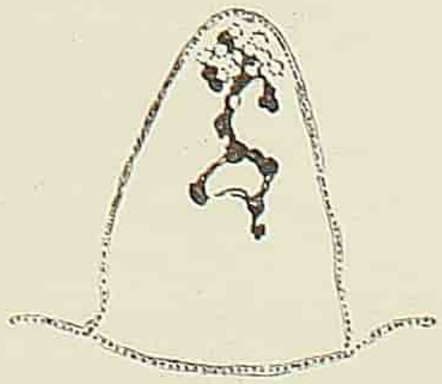
TABLE 1.

Pancreas of Rabbit No. 834

Time	Procedure	G Fresh tissue (Neutral red)
9:00 11:00 11:30	30 cc Neutral red solution injected *Tissue removed (control)* 10 cc Neutral red solution injected	 1
12:30 3:30	11 mg Pilocarpine injected *Tissue removed*	 5
4:00 5:00 7:00	11 mg Pilocarpine injected 20 cc Neutral red solution injected *Tissue removed*	 9
9:00	*Tissue removed* 6 cc pancreatic juice collected	 13

M, Mitochondria. Secretion granules indicated by dotted circles are unstained.
G, Golgi.

TABLE 1.

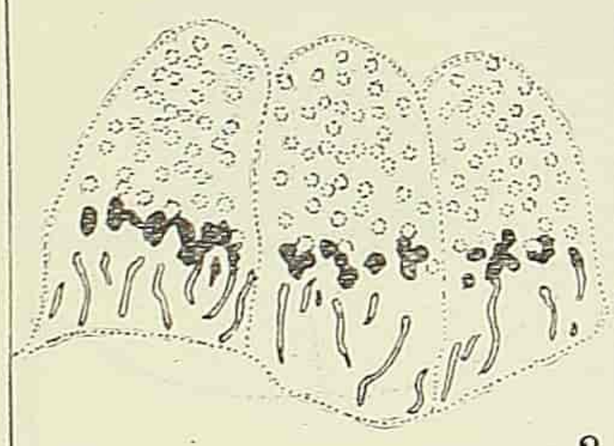
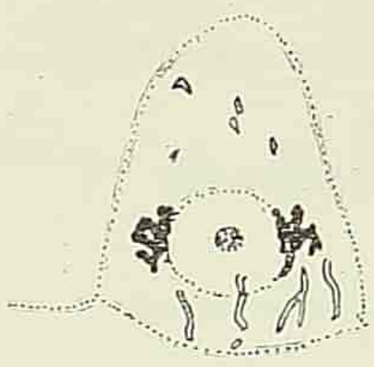
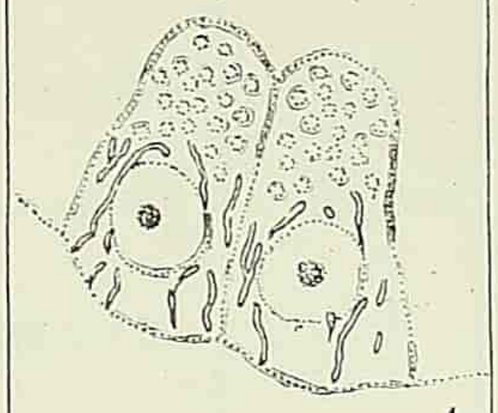
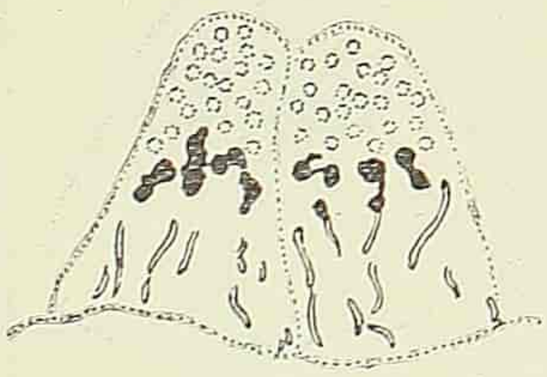
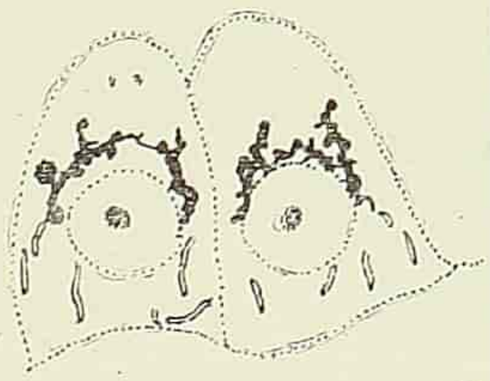
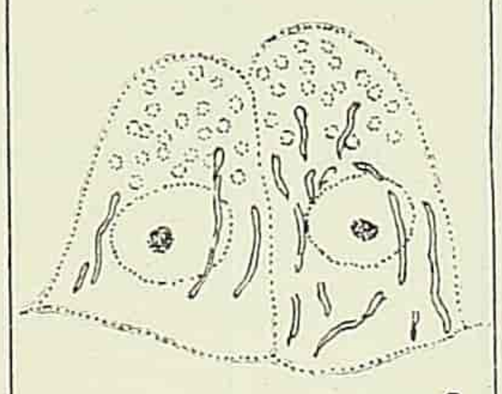
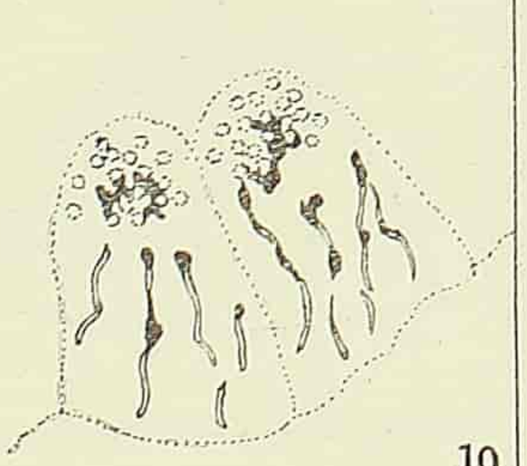
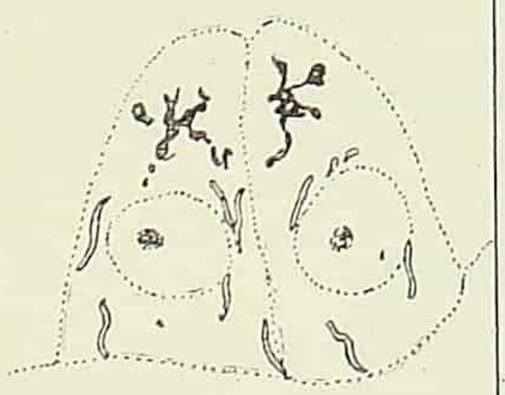
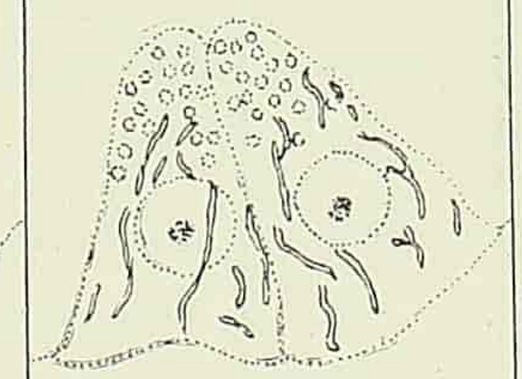
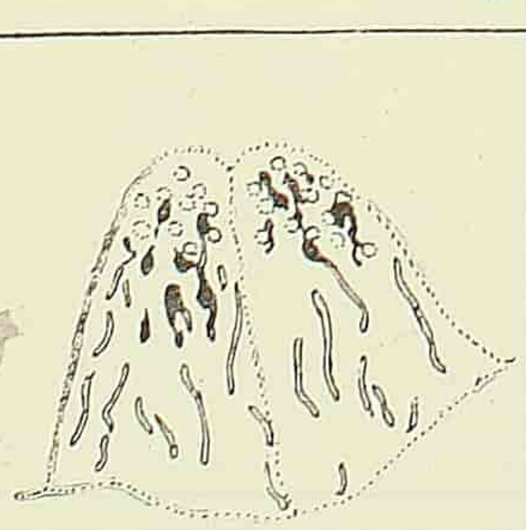
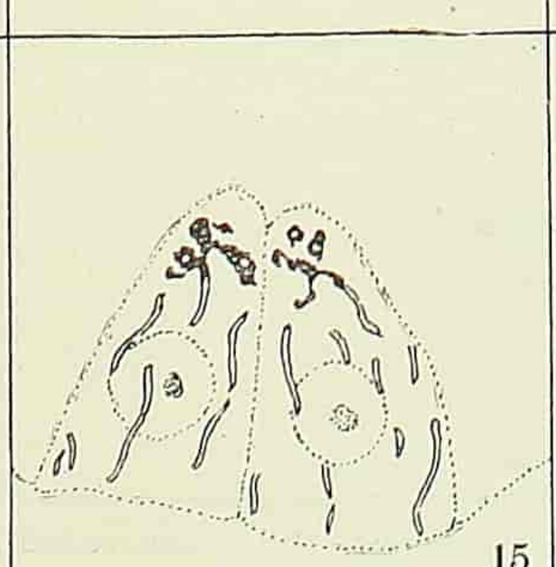
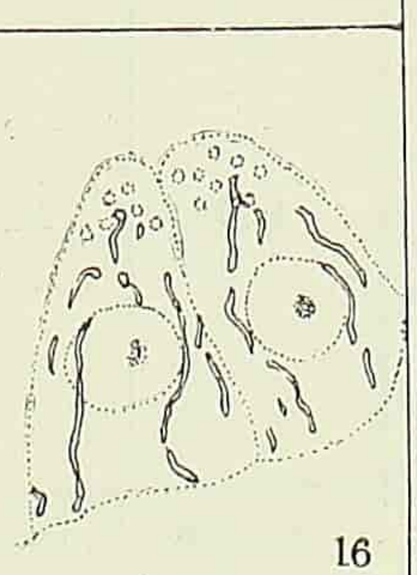
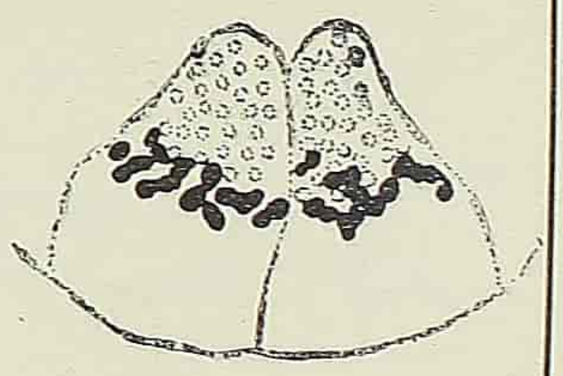
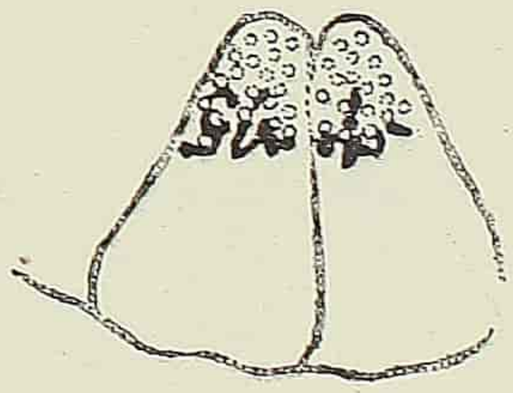
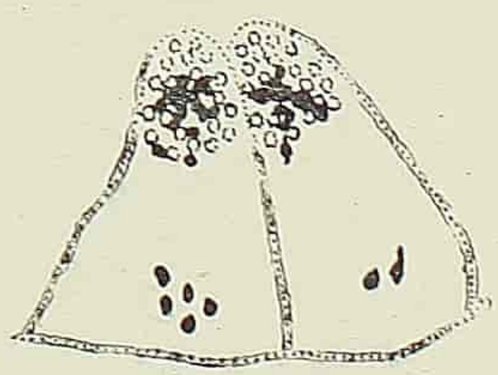
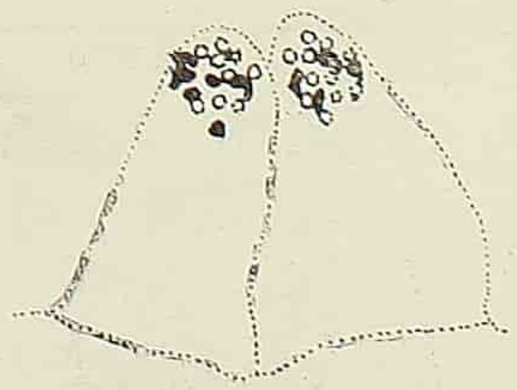
MG Fresh tissue (Neutral red-Janus green)	MG Fixed tissue (Osmic)	M Fixed tissue (Regaud)
 2	 3	 4
 6	 7	 8
 10	 11	 12
 14	 15	 16

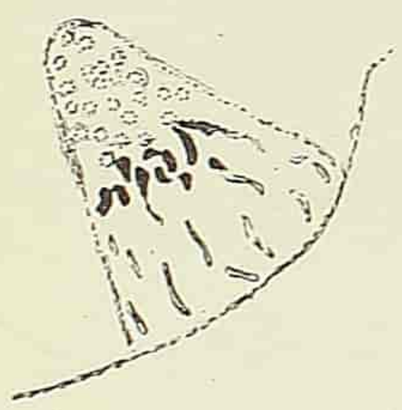
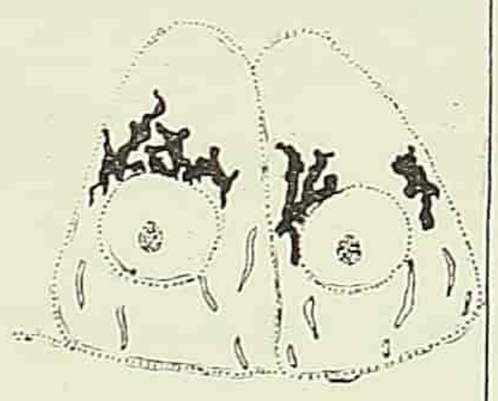
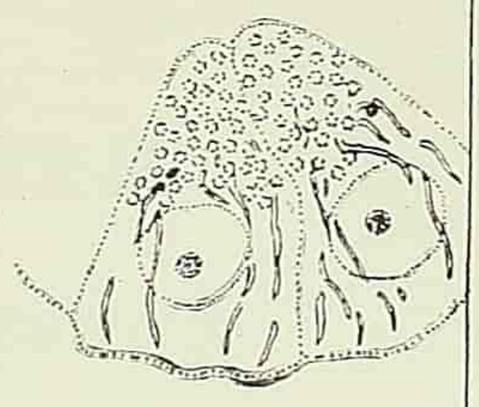
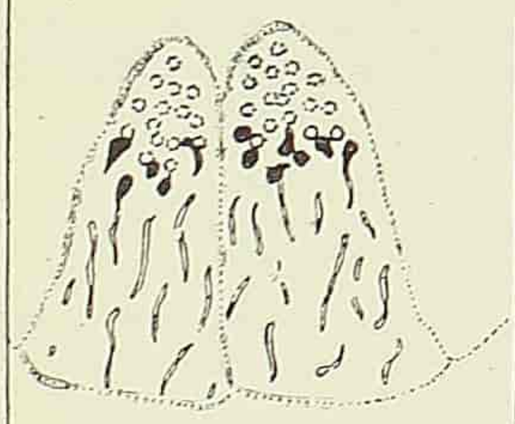
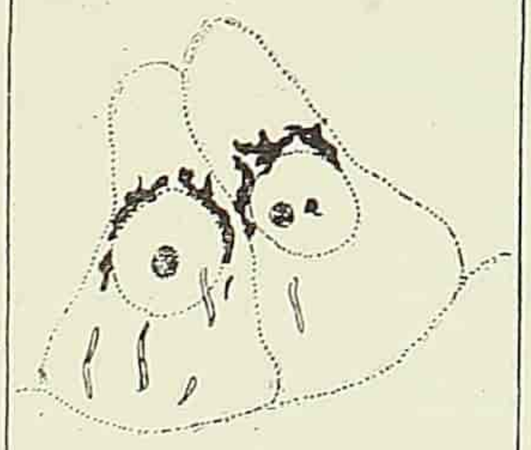
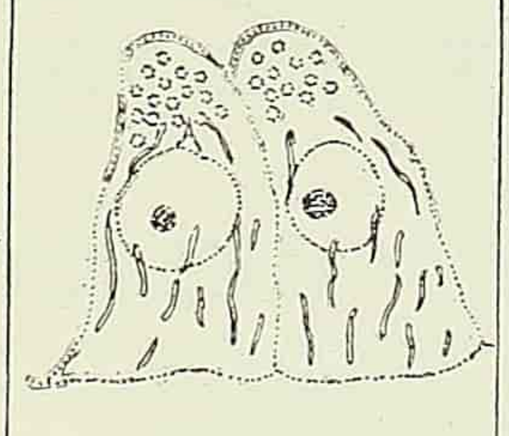
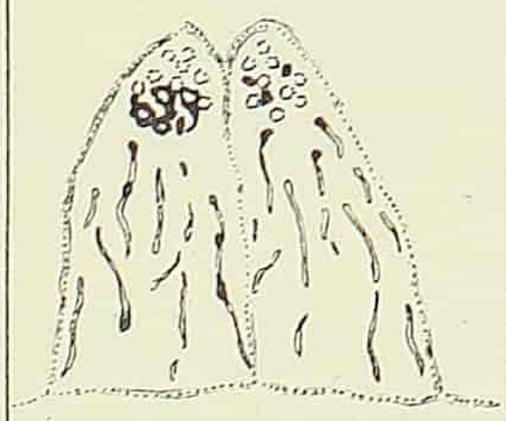
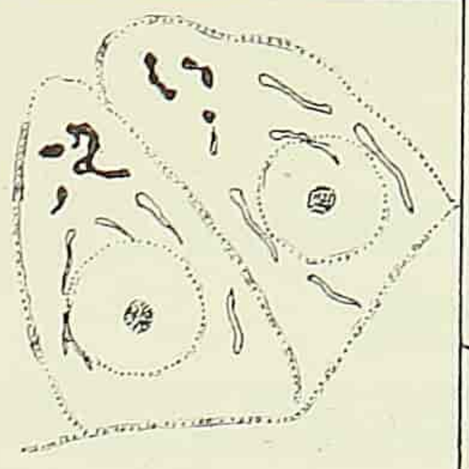
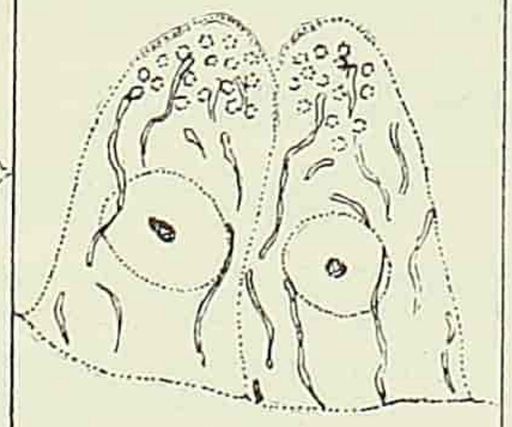
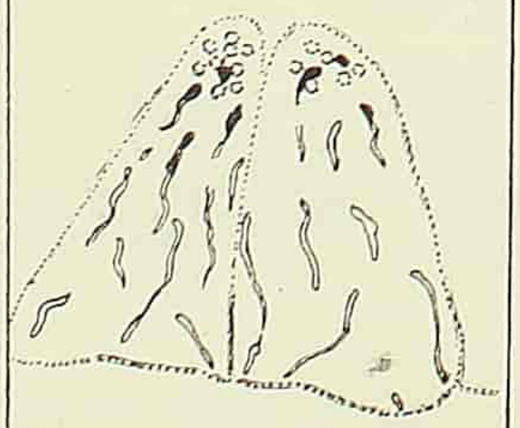
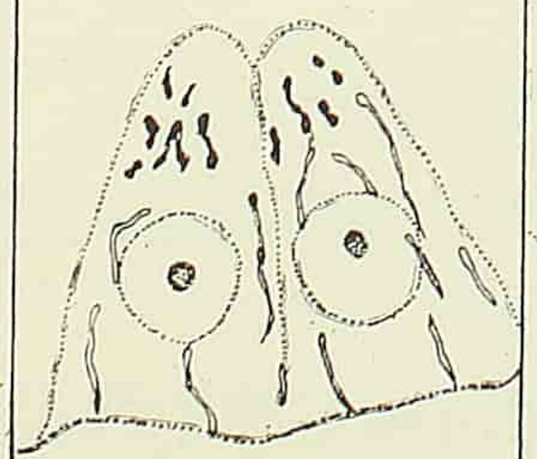
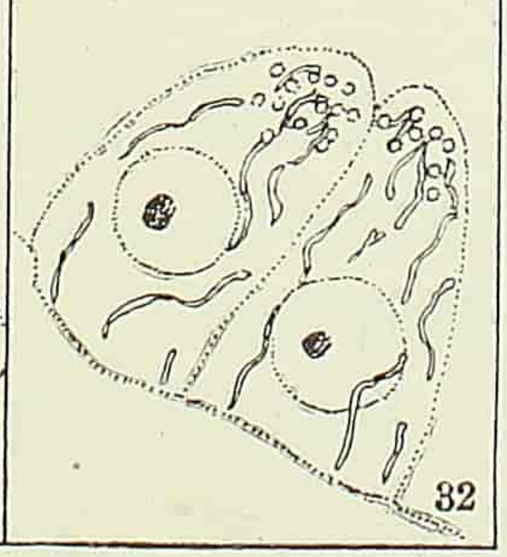
TABLE 2.

Pancreas of Dog No. 110

Time	Procedure	G Fresh tissue (Neutral red)
11:30 11:30 11:45	160 cc Neutral red solution injected *Tissue removed (control)* 22 mg Pilocarpine injected	 17
11:45	*Tissue removed*	 21
1:45 2:00 2:45 3:00	11 mg Pilocarpine injected 30 cc Neutral red solution injected 30 cc " " " " *Tissue removed* 11 mg Pilocarpine injected	 25
4:20	*Tissue removed*	 29

M, Mitochondria. Secretion granules indicated by dotted circles are unstained.
G, Golgi.

TABLE 2.

MG Fresh tissue (Neutral red-Janus green)	MG Fixed tissue (Osmic)	M Fixed tissue (Regaud)
 18	 19	 20
 22	 23	 24
 26	 27	 28
 30	 31	 32

分泌與"線高複體"之關係

其三。從生理作用上指認活體內染成之高基氏器

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用紅色染料(Neutral red)及蘇但第三，於活體中染高基氏器法，前此已詳論及之。本篇簡稱之爲"染高器新法"。

本篇從生理作用上證明所染者確爲高基氏器。

高基氏器之位置隨細胞工息而轉移。細胞靜止時，高基氏器稠密位於細胞核之下。細胞起生理動作時，高基氏器則變鬆散而近於細胞之腔端。此種變象可用鐵酸飽合法表出之。

本篇研究胰腺及唾液腺之細胞，以鹽酸疋羅卡品爲興奮細胞動作之劑。

注射鹽酸疋羅卡品之前後細胞中，高基氏器之位置，用染高器新法染出者，與用鐵酸飽合所染者適相符合。

蘇但第三能溶於紅色染料中，而紅色染料則能與脂質物溶解，此其所以能染高基氏器也。(按高基氏器爲一種脂質物)

高基氏器之能由細胞腔中洩出，於本篇中復提及之。

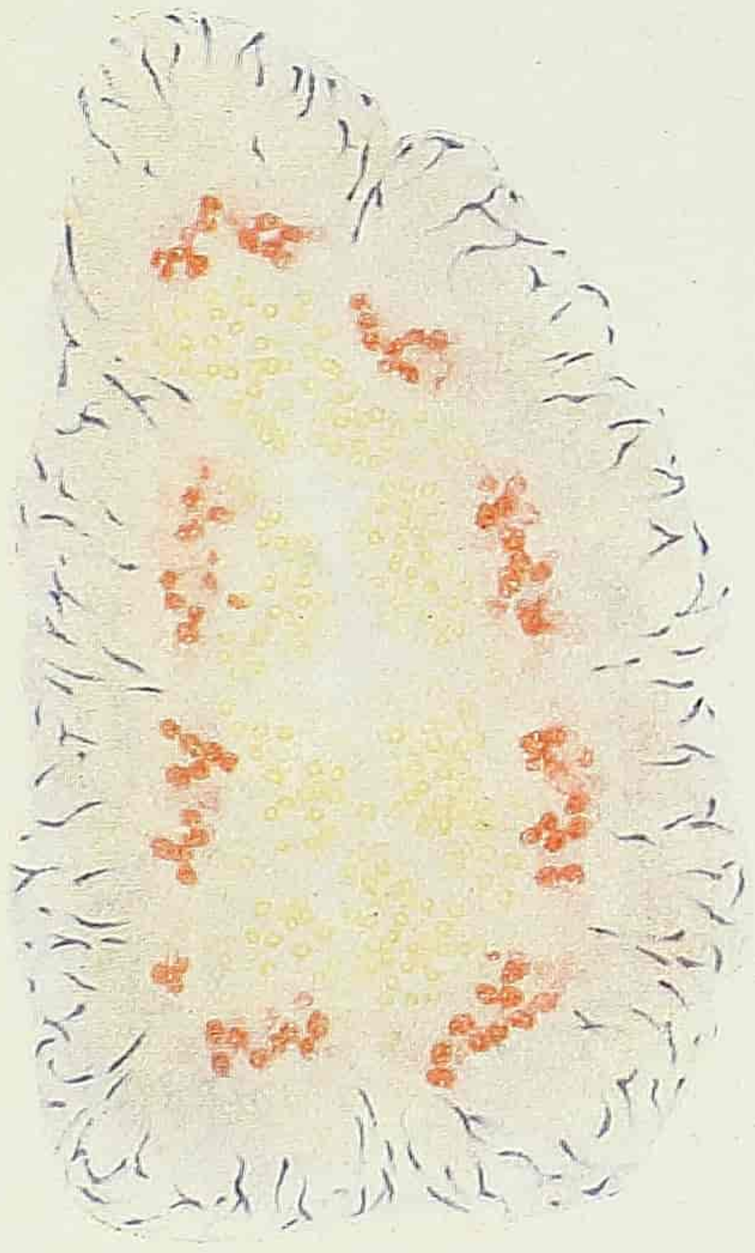

Fig. 33.

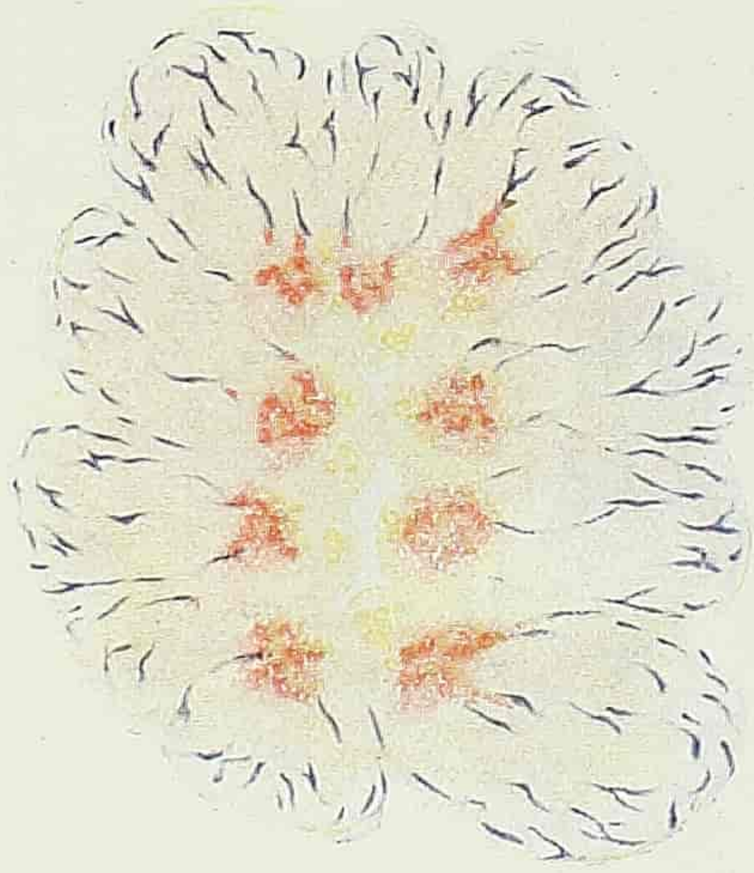

Fig. 34.

Coloured drawings of mouse pancreas intravitally stained by neutral red-Sudan III and supravitally stained by Janus green.

Fig. 33. Control. Note "granular" appearance of neutral red-Sudan III stained material.

Fig. 34. 5 hours after injection of 5 mg of pilocarpine.

Figs. 33–34. The Mitochondria-Golgi Complex.

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THE BASAL SECRETION OF THE STOMACH
II. THE INFLUENCE OF NERVES AND THE QUESTION OF SECRETORY "TONE" AND REACTIVITY

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The influence of the vagus on gastric secretion was first demonstrated by Pavlov (15) who improved the technique of the operation for the Heidenhain gastric pouch so as to preserve the vagal fibres.

The Pavlov-pouch is more reactive and secretes more copiously than the Heidenhain-pouch for this one reason. Orbeli (14) proved the corollary, namely that when a Pavlov-pouch is converted into a Heidenhain-pouch by severing the connecting muscular bridge, containing the vagal and enteric nerves to the pouch, the secretory response of the pouch to meals becomes much diminished. Later, Litthauer (11) and Rabinkova (17) showed that after double vagotomy above the stomach, the secretion of the "nüchterne" Pavlov-pouch occurs continuously though varying in amount. Savitsch (18) found that by dividing the stomach completely across between fundus and pyloric antrum, the former secreted spontaneously, but if the mucosa alone was divided, leaving the nerves and the muscle layers intact, the fundus remained inactive. Even the imposition of a gastero-enterostomy will according to Brestkin (2) give rise to hypersecretion (presumably spontaneous fundus secretion).

Although Pavlov and Schumova-Simanovskaja (16) had shown that the cephalic phase of gastric secretion involves the vagi alone (a fact recently reconfirmed by Farrell, 4), Volborth and Kudryzvzev (20) have drawn attention to the possibility of exciting a small gastric

response by electrical stimulation of the splanchnic nerve, in which the vasomotor fibres have been caused to degenerate by section 5 days previously. This would indicate that secretory fibres to the stomach travel in both vagus and sympathetic nerves. The existence of inhibitory fibres in the vagus has been demonstrated by Pavlov and his school, and in the sympathetic by Ishido (5), although the inhibitory effect (on meal stimulation) is slight. Except for the inhibitory effect of the vagus, the influence of nerves on the basal secretion is not definitely known.

METHODS

The following types of gastric preparations have been employed. All pouches consist of about one-sixth of the whole stomach.

1. *Fistula-pouch.* This is a modified Pavlov-pouch constructed as follows. A longitudinal incision about 5 cm long is made in the ventral wall of the stomach in the position indicated in fig. 1. The gastric cavity opened and the mucosal incision carried round the stomach along a line parallel to the fundic part of the lesser curvature. The cut edges of the mucosa are then sutured so as to form two separate gastric cavities, one in normal continuity with the rest of the alimentary tract and the other completely shut off. This outer chamber is fistularized. In this preparation all nerves are preserved and there is a minimum of injury to the enteric nerve plexus.

2. *Reverse-Pavlov-pouch.* This differs from the usual Pavlov-pouch in that it is made in the reversed way. Instead of making the cut in the stomach from the pyloric region towards the cardiac end, the greater curvature is incised from the cardiac dome towards the pylorus (see fig. 2). In this way it was hoped to have a preparation lacking only in vagal fibres and retaining the enteric and sympathetic connexions.

3. *Pavlov-pouch.* (See 9, fig. 2).

4. *Heidenhain-pouch.* This pouch has neither vagal nor enteric connexions, but again some fibres from the dorsal vagus may be present, if the pouch is constructed from the upper fundus region (see 9, fig. 3).

5. *Auto-transplanted-pouch.* This is a Heidenhain-pouch cut away entirely from every connexion and replanted by vessel anastomosis (see fig. 3). It is of course completely denervated, but contains the Auerbach and Meissner plexuses included within its walls.

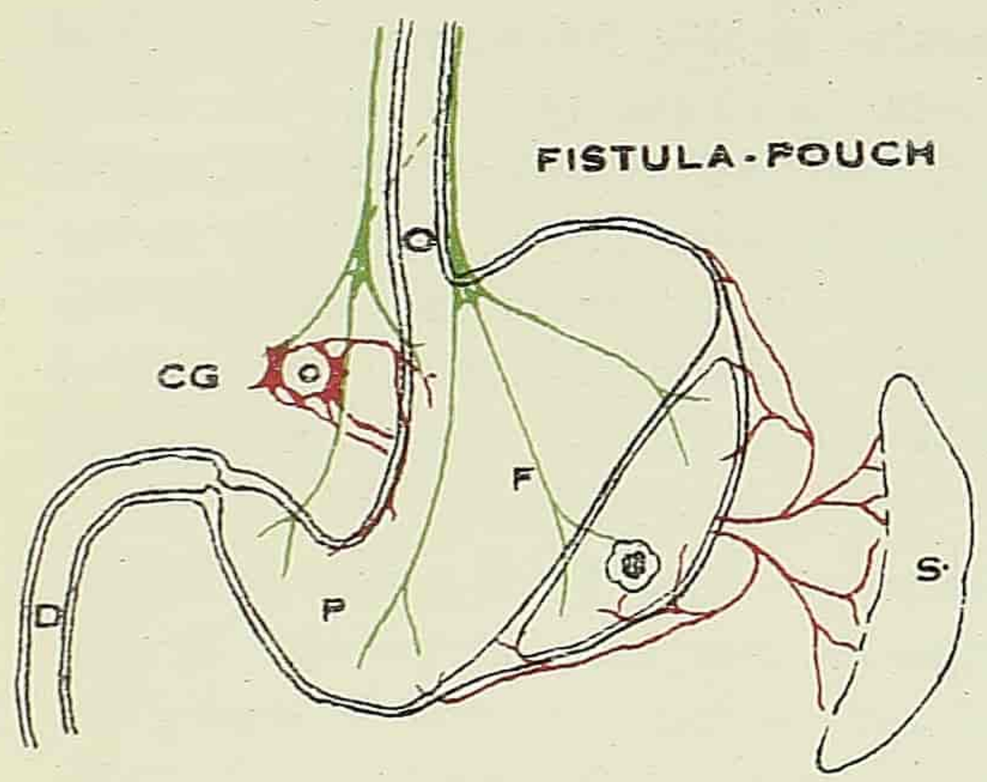

Fig. 1. Fistula pouch. *Green*, vagus: *Red*, sympathetic: CG, coeliac ganglia. O, oesophagus; F, fundus; P, pylorus; D, duodenum; S, spleen.

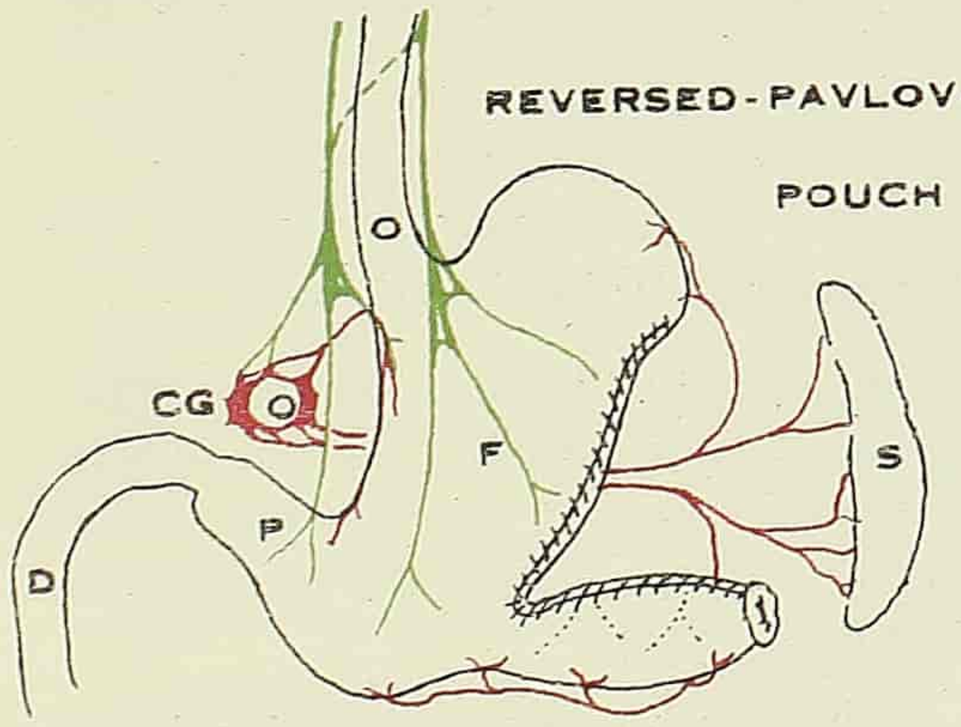

Fig. 2. Reversed – Pavlov-pouch. The dotted lines here and in fig. 3 indicate degenerated nerve fibres.

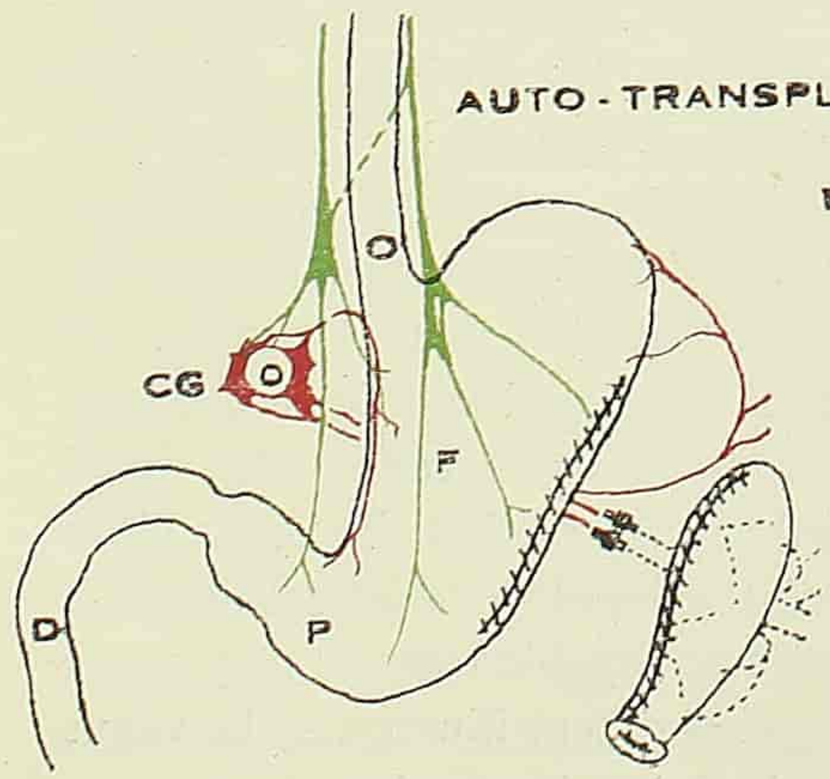

Fig. 3. Auto – transplanted-pouch. Note the site of vessel anastomosis and that the spleen is removed.

The diet consisted of a morning meal of 200 gm raw chopped beef and 100 cc water sometime between 10 and 11 a.m. and an evening meal of 300 gm of cooked corn meal and soy-bean flour (the former constituting two-thirds of the gruel) between 4 and 5 p.m. This regime was strictly adhered to during the week, but on Sundays the gruel was given at 12 p.m. with some amount of green vegetables. One litre of water was left with the animal from 4 p.m. to 8 a.m. the following morning.

Secretion was collected for two or three hours before giving the morning feed, and was continued for four hours afterwards. Several special procedures were applied but the secretion collected during and some days following these periods are not included in the present study. The figures given for the basal HCl secretion are the average of two hourly periods, while those for the meal response are the average of the first four hours.

RESULTS

The frequency and range of acid secretion under basal conditions.

In the fistula-pouch, the frequency distribution is almost equally divided between the secretory groups given in table 1, and there is no instance of a rate above 20 mg HCl per hour. Thirty-one per cent of the rates observed fall below 1 mg HCl per hour, while the remainder is fairly evenly spread between 1 and 20 mg HCl.

In reversed and "non-reversed" Pavlov-pouches, secretion below 1 mg HCl per hour occurs more frequently (47 per cent) than in the fistula-pouch, while the frequency at higher rates falls off proportionately, although 3 per cent occur above 20 mg HCl per hour. The close similarity of the mean figures for the Pavlov-pouches is noteworthy for they differ in that one (the reversed) lacks either entirely or to a very great part vagal innervation; the difference between the fistula- and the simple Pavlov-pouch is purely quantitative in that the former is larger and retains a greater number of vagal and enteric connexions. Since no significant difference seems to exist between the basal behaviour of those pouches, we are lead to suspect that the vagus plays no particular rôle in the *maintenance* of the basal secretion (although it is admitted that unrecognised conditioned or psychic reflexes may occur under basal conditions). The following experiment illustrates the vagus influence over the basal secretion in an individual animal.

TABLE 1.

Percentage frequency of acid secretion and its range under basal conditions in various gastric preparations

Dog	No. of daily observation	Secretory rates (mg HCl per hour)					Mean basal	Mean active
		0-1	1-5	5-10	10-20	20+	HCl *mg per hr.*	HCl *mg per hr.*
(1) Fistula-pouch								
342	6	67	19	7	7	0	2.6	40.1
365	8	38	50	12	0	0	2.2	29.8
392	4	0	2	25	73	0	14.0	86.8
573	5	20	30	40	10	0	5.5	19.4
Mean *		31 *35±10*	25	21	23			
(2) Pavlov-pouch								
5 dogs *	451	47 *42±2.3*	34	10	6	3	—	—
(3) Reversed-Pavlov pouch								
512	6	66	16	15	0	0	2.4	68.0
519	9	72	5	5	13	5	3.0	30.6
530	5	30	40	10	20	0	5.0	36.1
559	11	68	23	9	0	0	1.5	7.7
595	13	31	53	16	0	0	2.2	16.2
Mean *		47 *53±7.5*	34	11	7	1		
(4) Heidenhain-pouch								
114	42	55	45	0	0	0	—	—
626	22	82	18	0	0	0	0.7	9.7
686	15	67	33	0	0	0	1.0	11.1
726	13	85	15	0	0	0	0.5	5.2
Mean *		72 *68±4.9*	28					
(5) Auto transplanted-pouch								
568	122	85	15	0	0	0	0.5	3.9
626	40	53	33	10	4	0	2.4	17.3
686	40	95	5	0	0	0	0.3	15.3
726	64	86	11	3	0	0	0.8	6.0
Mean *		80 *82±2.4*	16	3	1			

* The figures in italics in column (0-1) give the percentage with the mean error of each group regard less of individual frequencies.

Dog 595 with a reversed-Pavlov-pouch was observed during the months of September and October. On October 25, both vagi were divided just below the diaphragm, and the basal secretion followed after the vagotomy. The animal died suddenly on the tenth day, when an autopsy showed a dilated stomach, a spastic pylorus and some congestion of the lungs.

The influence of the vagotomy on the secretion is evident from table 2 and fig. 4; while the basal secretion increased in range, the response to meals became much diminished (although the animal took its usual meat-meal greedily—to its own undoing!). The marked reduction in the response to meals may be explained by supposing that the vagotomy delayed gastric digestion and thus the liberation of secretagogues from food.

The increase of basal secretion is hardly significant but supports Litthauer's statement that double vagotomy causes a continuous (increased) though irregular basal secretion.

An interesting counterpart to the last experiment is supplied by the observations on dog 559, which also began with a reversed-Pavlov-pouch. After noting the nature of the basal secretion during the month of August, the muscular bridge connecting pouch and main stomach was divided converting the pouch into a Heidenhain one. From table 2 and fig. 5, it will be seen that the basal secretion diminished in range, but the meal response remained unchanged.

TABLE 2.

Percentage frequency of acid secretion in reversed-Pavlov-pouch dogs before and after vagal and enteric neurotomy

Dog	Remarks	No. of daily observation	Secretory rates (mg HCl per hour)					Mean basal HCl	Mean active HCl
			0–1	1–5	5–10	10–20	20+		
RP–595	Before	13	31	53	16	0	0	2.2	16.2
	After vagotomy	7	14	64	14	8	0	3.3	5.4
RP–559	Before	11	68	23	9	0	0	1.5	7.7
	After conversion to Heidenhain	13	96	4	0	0	0	0.4	7.1

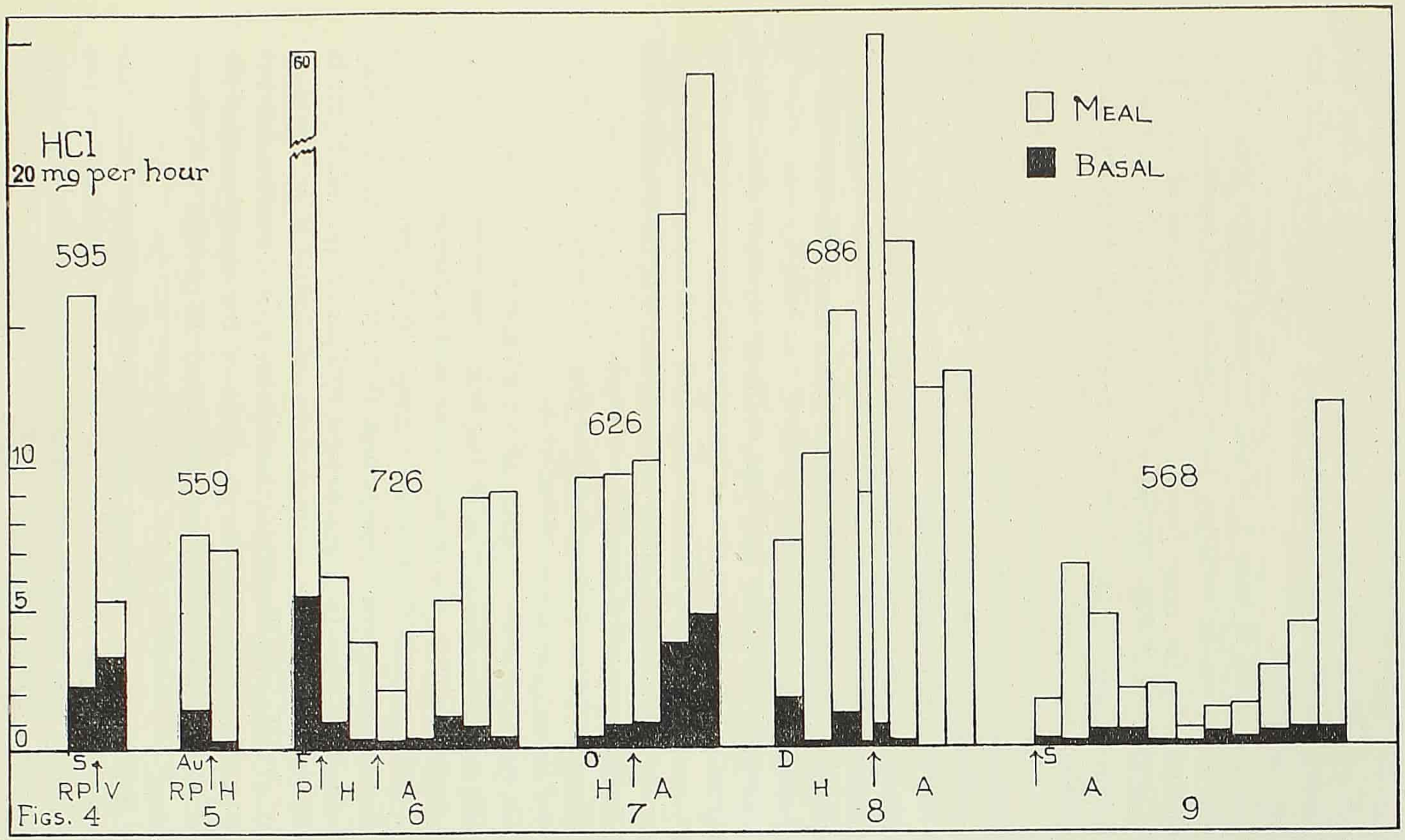

Fig. 4-9. The basal (black columns) and meal (white columns) secretion of innervated and denervated gastric pouches. RP, Reversed-Pavlov-pouch; P, Pavlov-pouch; H, Heidenhain-pouch; A, Auto-transplanted-pouch; V, double vagotomy. The arrow indicates the time of operation. Each column represents a month but end columns may represent less than one month (the first letter of the first month is given in each case, e.g. S = September).

Since the conversion from reversed-Pavlov- to a Heidenhain-pouch chiefly or only involves the enteric plexuses, it seems fair to allow that the diminution of the basal secretion should be attributed to the loss of enteric connexions.

Referring again to table 1, it will be noted that the Heidenhain-pouch has a still smaller secretory range (under basal conditions) than that exhibited by the three types of pouches described above. The range does not exceed 5 mg HCl per hour in the series observed, while more important still is that about 72 per cent of all observations show less than 1 mg HCl secretion.

In the auto-transplanted (completely denervated) pouch the greatest number of observations is also included in the groups below 1 mg HCl per hour (80 per cent), but the range is larger than in the Heidenhain-pouch there being records of basal secretion up to 20 mg HCl. The majority of the observations below 1 mg HCl, indicate either no acid secretion or so little that it may be taken as zero. The average basal secretion of the completely denervated pouch is not different from the average of the Heidenhain-pouch.

It may be mentioned here that the basal secretion of the Bickel (Heidenhain-pouch with section of post-ganglionic sympathetic fibres) and the coeliac-ectomised (Heidenhain-pouch with removal of the sympathetic cell stations, viz., coeliac ganglia) pouch is of the same order as that exhibited by the auto-transplanted-pouch.

The conclusion may safely be drawn that the sympathetic exerts no particular influence on the basal secretion.

Now, this being so and granting that the vagus may possibly inhibit the basal secretion, comparison of the first three groups with intact enteric connexions, viz., the fistula, the reversed and the normal Pavlov-pouches with the Heidenhain- and the completely denervated pouches, which have no enteric connexions, shows that not only is the frequency of low secretory rates (i.e., below 1 gm HCl per hour) much higher in the latter than in the former, but that the order of the frequency alone (viz., 31–47 versus 72–80) divides the various types of pouches into two natural groups. There can be no doubt of the significance of the difference between the auto-transplanted and the pouches with enteric connexions.

It is thus clearly indicated that the enteric plexuses constitute what is equivalent to a "conducting" or perhaps "co-ordinating" system such that any part which is cut off from the system no longer keeps pace with the activity of the main organ.

In the normal stomach with intact nerves, the vagal influence may dominate the enteric, but whether their actions are directly antagonistic or exerted on different phases of the gastric secretory mechanism is not clear. The fact that atropine diminishes or abolishes the basal secretion (7,9) shows that a nervous factor is present, but whether the effect is obtained by stimulating the vagal endings or paralysing the enteric we do not known.

Changes in the basal secretion following successive and/or complete denervation.

The effect of successive denervation in a single animal throws further light on the problems discussed above.

Dog 726, was first provided with a Pavlov-pouch on February 5, 1927, and secretion observed on four occasions after recovery; at the end of 25 days, the pouch was converted into a Heidenhain. Observations were continued for a period of 48 days, when the pouch was transplanted so as to complete the denervation. The secretion of the transplanted pouch was followed for 152 days. On converting the Pavlov- into a Heidenhain-pouch the mean basal secretion fell from 5.4 (σ 2.4) to 0.5 (σ 1.2) mg HCl per hour, while after transplantation, there was some slight increase, 0.8 (σ 2.1). This accords well with the results of the group experiments given in table 1; further it will be noted that after complete denervation the variability increased (see fig. 6).

The response to meals suffered markedly after vago-enteric section, viz., 60.1 (σ 20.8) to 5.2 (σ 4.8) while after complete denervation, there was an insignificant improvement (6.0, σ 4.2). If the secretory change, however, is followed daily and the results grouped for convenience into monthly periods, it will be found that the Heidenhain-pouch showed a progressive deterioration of both basal and active secretions which continued for a brief period after complete denervation, but that later the basal secretion began to fluctuate between a larger range and the active secretion or response to meals recovered progressively so that by the fourth month it exceeded that of the Heidenhain-pouch stage. This animal died on November 3, 1927, 193 days after transplantation; the autopsy showed that the couplers used in the anastomosis of the gastric vessels were in a pus pocket surrounded by omentum, the original vessels having been completely absorbed. New vessels, however, were found in the peripheral adhesions attached to the transplanted pouch, and the question arises whether secretory nerves had arisen to supply the glands (see fig. 10).

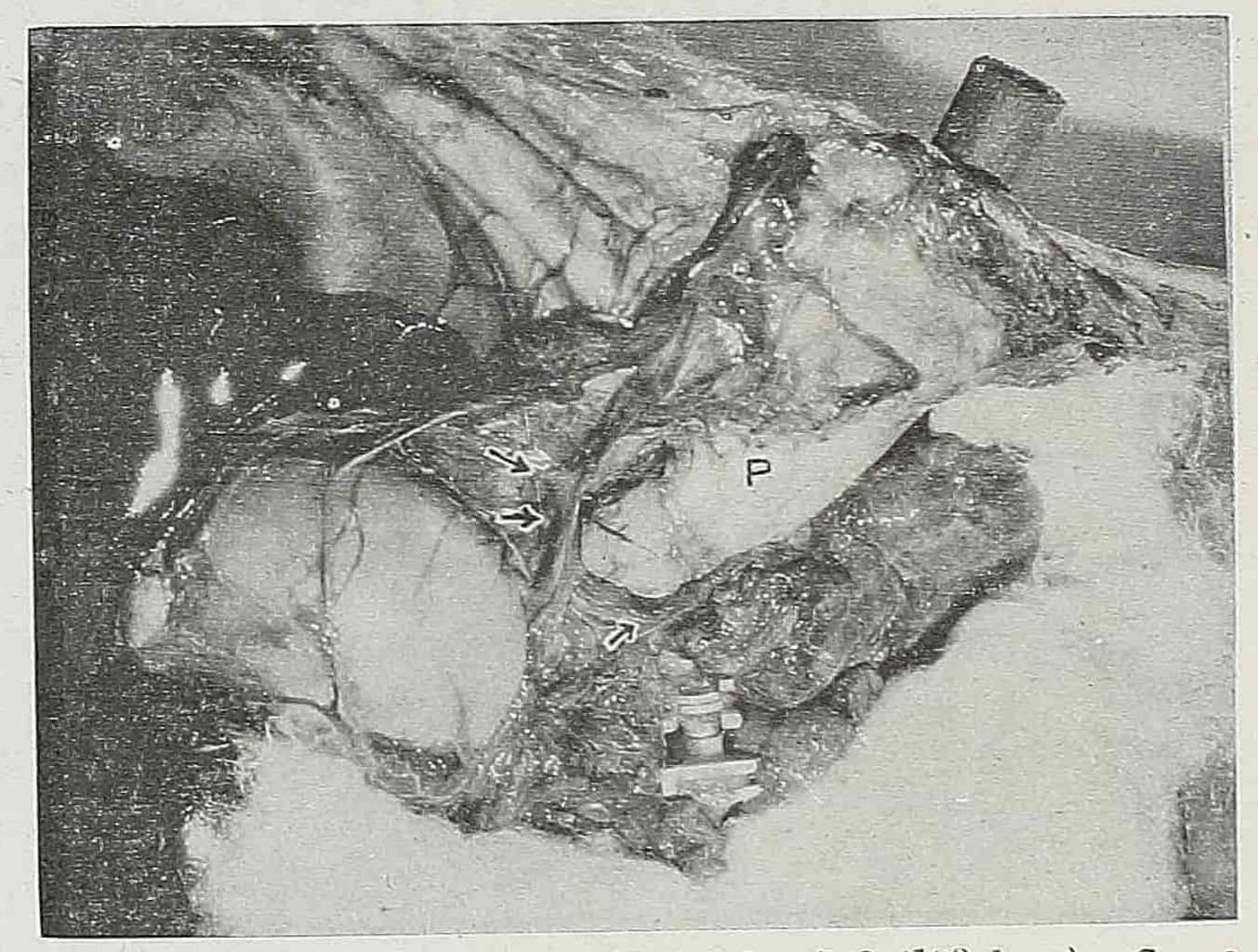

Fig. 10. Auto-transplanted-pouch (P) of dog 726 (193 days). Couplers lying below pouch completely freed from vessels. New vessels (indicated by arrows) may be seen between the pouch and the main stomach.

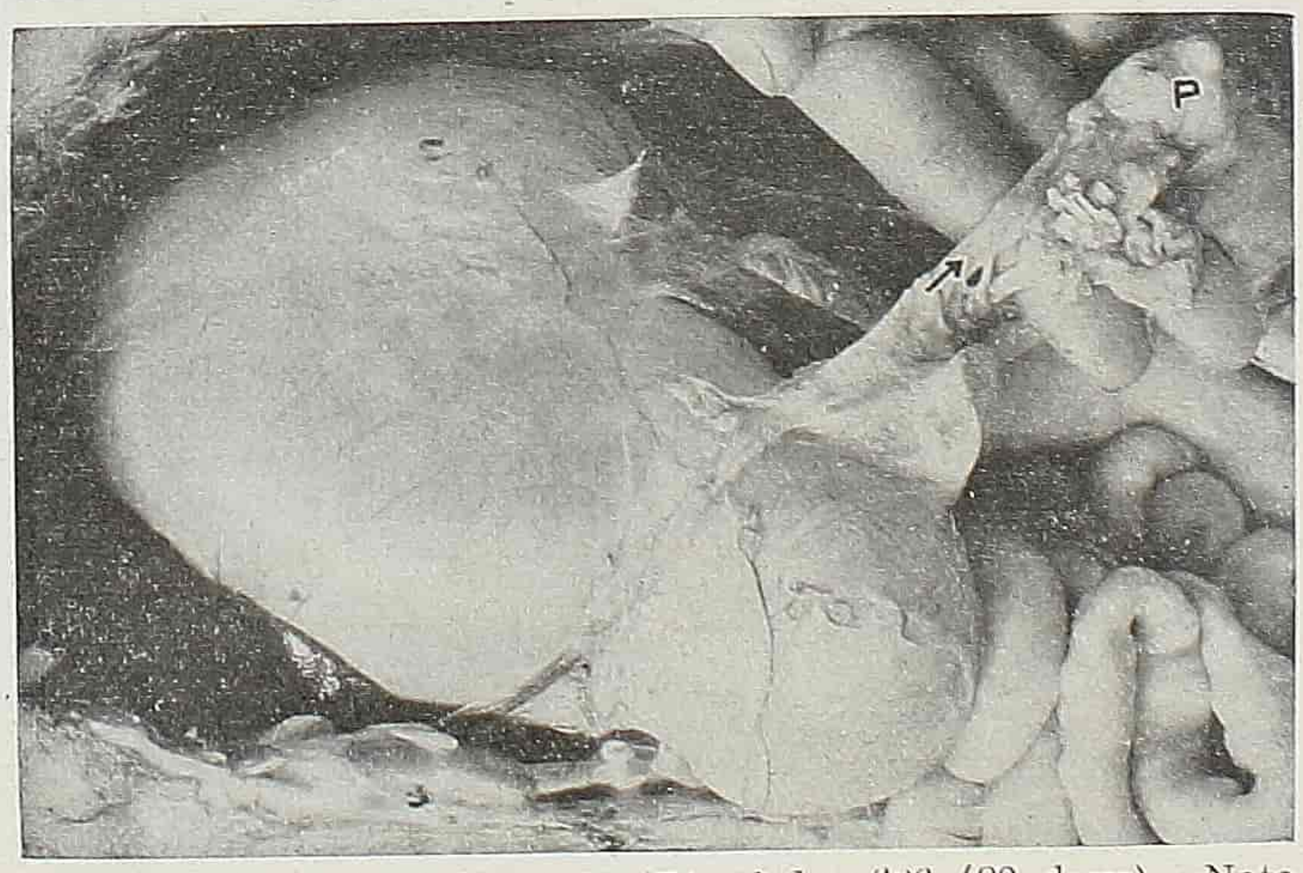

Fig. 11. Auto-transplanted-pouch (P) of dog 626 (90 days). Note coupler encrusted with calcium deposit and vessels still in alignment. New vessels are indicated by the arrow. The pouch is situated just above the couplers.

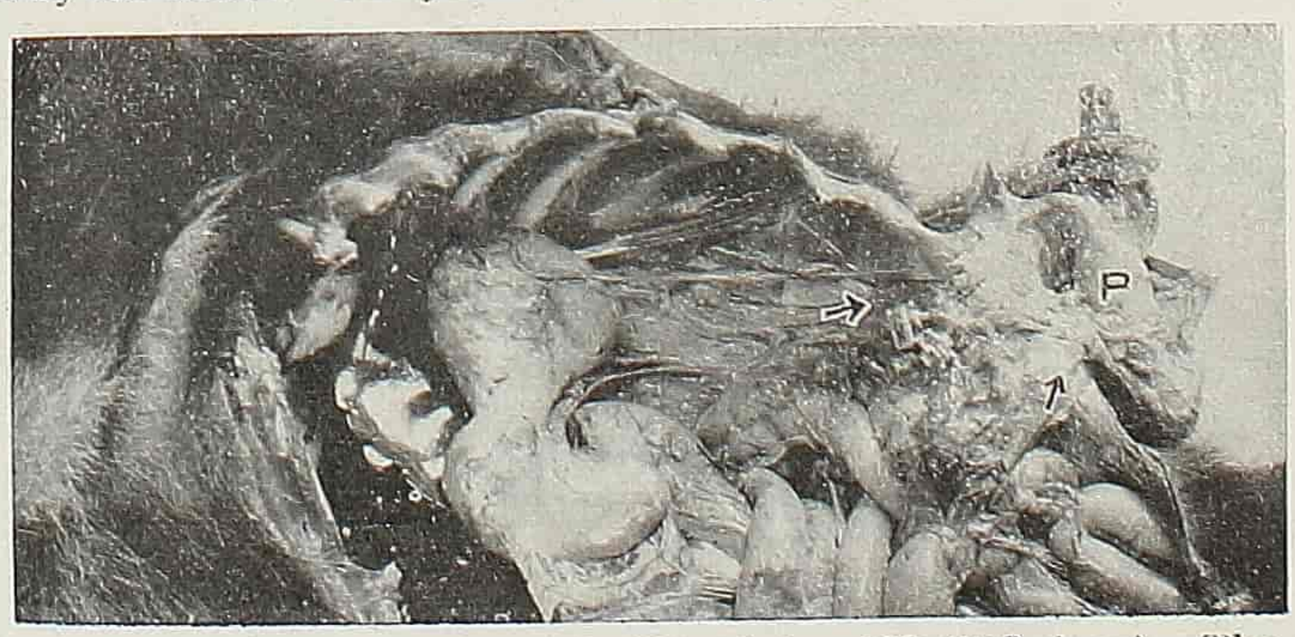

Fig. 12. Auto-transplanted-pouch (P) of dog 568 (358 days). The couplers with the original vessels are exposed; the vein is obliterated at the peripheral end. New vessels are indicated by arrows. Note hour-glass shape of main stomach.

Two animals with Heidenhain-pouches were followed before and after the transplantation of their pouches (see figs. 7 and 8). Dog 626 was provided with a Heidenhain-pouch on October 12, 1926; after 49 days the pouch was transplanted and observations continued for another 89 days. The basal secretion increased from 0.7 (σ 0.6) to 2.4 (σ 3.8), while the response to meals mounted from 9.7 (σ 5.0) to 17.3 (σ 7.4); the basal secretory range also increased after complete denervation. The animal died from pneumonia on February 28, 1927; at autopsy the couplers were found encapsulated by omentum, and surrounded by calcium deposits (see fig. 11); they were in proper alignment, but probably very little flow occurred through the vein as several new vessels, had developed between the omentum and the pouch.

Dog 686 was provided with a Heidenhain-pouch on December 4, 1926 and the pouch transplanted on March 11, 1927. The basal secretion deteriorated from the time the pouch was constructed but taking the mean value, a change from 1.0 (σ 1.2) to 0.3 (σ 1.6) was observed after complete denervation (see fig. 8). The response to a meal gradually improved from December 1926 to the time of transplantation; immediately after transplantation the response to meals increased remarkably but finally settled down to the level reached in February. The mean values before and after complete denervation was 11.1 (σ 5.1) and 15.3 (σ 8.0). This animal died of pneumonia on June 24, 1927, 105 days after the transplantation. The anastomosis were found in good condition, although surrounded by omentum and encrusted with calcium depositions. New vessels were present as in the other animals. Not unimportant was the finding of 2 rubber tubes 8.5 and 9.5 cm long in the stomach.

Dog 568 was provided with a transplanted pouch at the outset on August 27, 1926. During the first two weeks the pouch was refractory and it was thought that the transplant had not "taken". By the third week, however, the pouch had recovered and thereafter exhibited a low basal range of secretion with varying response to meat meals. There were two periods of heightened response, viz., October and November 1926 and during the last months of June and July 1927; in fact from the beginning of 1927 until the death of the animal in August, the basal secretion maintained a steady level of about 0.5 mg HCl per hour, while the response to meals increased gradually from practically no response (below 1 mg) in February to 12 mg in July (see fig. 9). These observations show that seasonal variations do not account for

the variation in the meal response for the lowest records were found in January and February, while in dogs 626 and 686, the maximum response to meals occurred in mid-winter. The animal died on August 20, 1927, having survived the transplantation of the pouch 358 days. At autopsy the pouch was surrounded by omentum and abundant new anastomotic vessels were present. The arterial anastomosis was patent but the vein had long been thrombosed and obliterated at the peripheral end (fig. 12). The pouch itself was in excellent condition. Pneumonia and multiple abscesses of the intestine were the cause of death.

In all four animals with gastric auto-transplants, new circulatory channels had established themselves. It is not unlikely that nerves accompanied the newly developed vessels, but it is extremely questionable whether they found their way to the gland cells or to the appropriate enteric reflex centres. The experience of others on the vago-sympathetic (Schafer, 19; Burlage, 3) certainly do not suggest that a successful functional regeneration of severed and particularly un-aligned autonomic nerves is to be expected, while Rabinkova (17) states that she could find no regeneration 1 year and 54 days after suturing the cut vagi. We are therefore inclined to view the improvement in secretory response which occurred as time went on as the result of a regenerated circulation rather than a recovery of nerve control.

The question of secretory tone and reactivity.

The changes observed on severing the enteric connexions are of interest chiefly because they show a diminution of the basal secretion without any significant alteration in the ability of the stomach to respond to meals (hereafter referred to as secretory reactivity). On the other hand, section of the sympathetics, and particularly the behaviour of the completely denervated pouches demonstrate a wide variability in reactivity but no characteristic change in the basal secretory rate. That the state of the basal secretion and secretory reactivity are independent of each other when meal stimulation is employed is clearly evident in dog 568. The basal rate in this animal during the months of March, June and July was 0.6 mg HCl per hour, but the response to meals during these months was 1.4, 4.4 and 12.0. Hence we can hardly speak of a basal secretory tone, for the state of the basal secretion does not predetermine the size of the response (to meals). It must be pointed out that a difference must be made between a direct stimulus (e.g., injection of histamine) and an indirect stimulus

(e.g., action of secretagogues in meals). With direct stimulation, the secretory response is an increase by a fixed proportion according to the level of the basal secretion, viz., $y=k+x$, where y is the response, k a constant and x the basal secretion. With indirect or meal stimulation the response cannot, as we have just seen, be predicted in this way. The reason for the difference must therefore be in the variability of the stimulating mechanism intervening between the indirect stimulant or secretagogue and the gastric cells.

Variability of the intermediary mechanism, however, may be complicated by alterations in the excitability or threshold of the secreting cells. We know for example that immediately after denervation (10) or following an injection of histamine (8) the threshold of the gastric cells is raised. It is possible that the converse, i.e., a lowered threshold may occur. In fact repeated subminimal stimulation appears to lower the gastric threshold (6). Variations in the threshold or excitability of the secreting cells may well be termed tone, but at the moment only conditions of depressed tone are known unless the clinical condition of hypersecretion is an example of increased excitability. The observations with histamine show that the tone of the glands is relatively constant under approximately standard conditions but do not exclude the possibility of increased tone under other yet physiological conditions. A raised secretory tone would permit stimulating factors (nervous or chemical) present in subliminal degree to act on the secreting cells, but tone does not in itself explain the basal secretion. Lastly tone must not be confused with reactivity, the former indicates the ability of the cell itself to respond to direct stimulation, while the latter indicates the ability of the entire gastric secretory apparatus (viz., receptive surface, intermediate mechanism and cell) to respond to indirect (meal) stimulation. Complete atony would prevent reactivity, but a non-reactive stomach might still respond to direct stimulation.

The present experiments show that secretion still occurs under basal conditions, although much limited in range, after all extrinsic nerves are divided. This reduced secretion must therefore be due either to the activity of the local enteric elements of the pouch or to chemical factors. Chemical excitants have been recovered from the circulation (13) under basal conditions and on occasions in amounts sufficient to account for a high basal secretion. Now these excitants must arise from either the stomach or intestine. We know that marked and even absolute delay of the total food residues in the small intestine

does not necessarily increase the basal secretion (12), and although this observation does not prove that the presence of normal amounts of residue in the intestine is not able to stimulate any secretion, it suggests that a more efficient chemical factor must be sought for elsewhere and particularly in the stomach. Whether the humoral excitants are spontaneously increted or are related to food residues remains to be determined, although the latter appears the more probable.

SUMMARY

1. Observations on the secretory behaviour of variously denervated gastric pouches have shown that with the vagus intact there is a greater variability of the basal secretion. Severance of the enteric connexions between the pouch and main stomach results in a diminished basal secretion, indicating that the integrity of these connexions assist the maintenance of the basal secretion. The sympathetic exerts no appreciable influence. In fact secretion still occurs under basal conditions after all extrinsic nerves have been divided.

2. The gastric response to meals cannot be predicted from the level of the basal secretion, as meal stimulation is indirect, involving motor activity (emptying time), and humoral and nervous secretory mechanisms. A distinction must therefore be made between the response to direct stimulation, e.g., by histamine or gastrin injections, and the response ("reactivity") to meal or indirect stimulation.

3. There is at present no evidence to indicate, either that a high basal secretory rate is due to the existence of a raised secretory "tone", or that changes in tone are necessarily reflected in the basal secretory rate.

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胃的基礎分泌。其二。神經的影響及分泌"調節"和反應性的問題

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觀察各種除去神經的小胃的分泌，得下列幾個結論：（一）迷走神經除習知的刺激功能外，尚有約束基礎分泌的作用。（二）小胃和主胃間的內神經連絡割斷後，小胃的基礎分泌減少：此種連絡的存在必有關于基礎分泌的維持。（三）交感神經對于胃的基礎分泌無影響。

胃分泌對于飼食的反應的大小，不能由基礎分泌的多少而預斷；因爲飼食的刺激是間接的，同時幷有胃動，情形複雜，非直接刺激如組織毒（Histamine）和胃分泌刺激物的注射所引起的反應可比。

關于胃的分泌"調節"問題，現在尚無證據可以證明高的基礎分泌率定由于分泌"調節"的提高，或分泌"調節"的變化必因基礎分泌率反應而來。

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FURTHER STUDIES ON THYROID AND HAIR GROWTH*

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That the thyroid gland has a specific influence upon hair growth has been proposed on the basis of the following observations (2). Feeding of thyroid extract to the albino rat accelerates hair growth in a number of individuals. Thyroidectomy besides decreasing body weight, retards hair growth. Partial starvation results not only in decrease of body weight but in retardation of hair growth and atrophy or regression of the thyroid (1). If, however, an adequate amount of thyroid extract is given to the *starved* animal, an acceleration of hair growth may be demonstrated in spite of insufficient anabolism from starvation and excessive catabolism secondary to thyroid feeding.

In the present study we have endeavoured to determine more precisely the nature of the thyroid effect and as to whether the hair response is a pure thyroid reaction, or one reproducible by some one of its chemical constituents. Incidently the factors of age and sex were further studied.

EXPERIMENTAL

Albino rats of the same age and sex (litter mates) were used for each experiment. A small square area was shaved on the right side of the back in the Chicago series, and on each side of the back in the Peping series. The number of days required for the completion of hair growth in the shaved areas was recorded. A reading of the normal rate of hair growth was obtained as a control in each experimental animal, shaving being done over the same area, for the successive readings.

*This work was started in the Hull Physiological Laboratory, Chicago, and continued in Peping.

Readings of the same side were usually compared. Milk and whole wheat flour constituted the control diet, fresh vegetable being given once a week. The general scheme of the experiment was as follows: After the first control reading, say in a series of four animals, three were fed a limited portion of the normal or control diet, while one was continued on the normal diet. Thyroid extract was given to one of the partial fed animals, iodine to the second and nothing to the third which served as a simultaneous control. Thus in each experiment, it was possible to compare the effect of thyroid (or iodine, etc.) with normal and partial feeding controls. The above scheme was more or less modified in different experiments according to the number of animals and substances tested. Besides thyroid siccum (Allen and Hanbury, and Armour) and iodine (Lugol's solution), 1 per cent aqueous tryptophane solution (Pfanstiehl) and 0.25 per cent tyrosine in N/100 HCl were studied. The solutions were administered by drops: 2 or 3 for iodine, 3 for tryptophane and 14 for tyrosine per day. During intervals of rest between readings all the experimental animals were on full diet.

RESULTS

In judging the influence of our experimental procedures upon the rate of hair growth, no rate was considered abnormal unless the deviation from the control was greater than three times the mean error of the series. The rate is said to be accelerated when it is faster than the mean rate by 3 E and retarded when it is slower than the mean by the same margin (see table 1).

Regional difference. In many animals, readings were taken from both sides of the back. The rate of hair growth of the two sides may not be the same in individual cases, but when the entire series is analysed, the mean rate of hair growth is found to be about the same for both sides.

	No. of observations	*Mean*	σ	E
Right side	96	30.7	12.6	1.3
Left ,,	100	28.8	10.6	1.6

Sex. No sexual difference in the rate of hair growth was observed in the present study (table 1 and fig. 1).

Age. Hair grows slowly during the first 44 to 65 days after birth, while from 71 to 260 days the rate of hair growth is faster (table 1), indicating that the age factor is not significant except in very young and possibly very old animals.

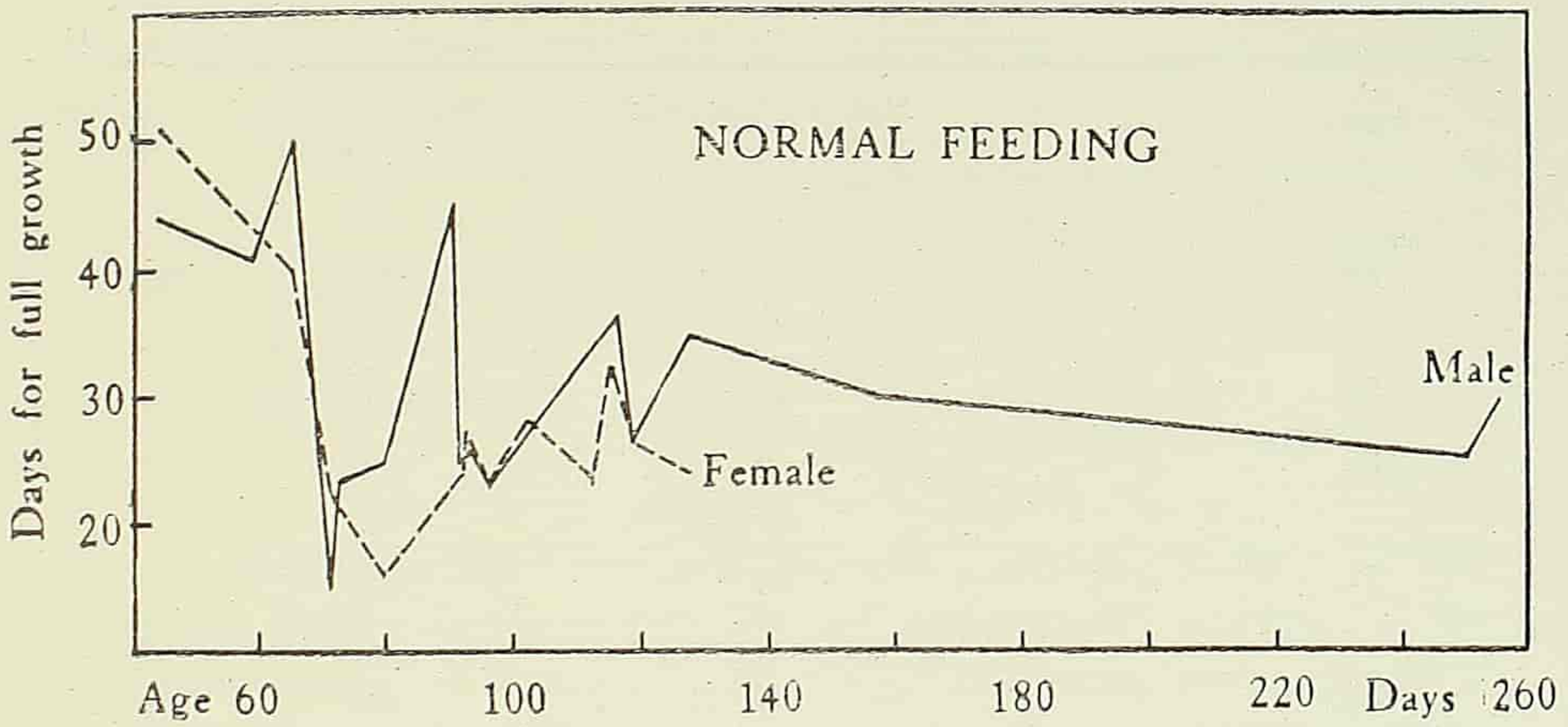

Fig. 1. Graph showing the influence of sex and age on the rate of full hair growth in albino rats on normal diet.

Partial feeding. As will be seen from the percentage distribution of the hair growth rates in normal and under-fed animals, under-feeding definitely retards hair growth.

	Acceleration (mean $-3E$)	*Normal rate* (mean $\pm 3E$)	*Retardation* (mean $+3E$)
I. Normal feeding	20 (34)	65 (107)	15 (26)
II. Partial feeding	0	46 (19)	54 (22)

(The figures in parentheses give the number of observations.) Whereas 15 per cent of normal animals have hair growth rates exceeding the normal mean $+3E$ (26+6 days), 54 per cent of partially fed animals have retarded rates. The other groups are affected accordingly.

It is obvious that even with normal feeding considerable individual differences in hair growth exist. To what are these differences due? The most probable factors are either nutritional or endocrine, and we believe that these are related and that the particular internal secretion involved is the thyroid.

The distribution of the partial feeding rates compared with the mean partial feeding rate (41±9, days) is as follows.

	Acceleration	*Mean partial feeding rate*	*Retardation*
III. Partial feeding	34 (14)	37 (15)	29 (12)

TABLE 1.

The influence of age and sex on hair growth

Age	Sex	Rate of full hair growth (average)	Number of observations	*E*
days		*days*		
44	♂	44	4	2.7
	♀	51	8	1.7
59	♂	41	7	3.9
65	♀	40	4	0.6
	♂	50	5	3.3
71	♀	23	9	1.2
	♂	15	5	0.4
72	♂	23	10	1.8
80	♂	25	10	3.1
	♀	16	6	0.7
90	♂	45	3	7.7
91	♂	25	18	1.1
92	♀	24	4	1.1
93	♀	27	12	1.6
	♂	26	8	1.1
96	♀	28	8	1.2
	♂	23	18	0.5
102	♀	28	3	8.0
112	♀	23	6	0.8
116	♂	36	8	2.8
	♀	33	8	2.1
118	♂	26	4	1.2
	♀	26	7	0.4
127	♀	24	6	2.0
	♂	35	6	3.3
158	♂	30	8	1.8
250	♂	25	7	1.0
255	♂	29	2	1.8

Thyroid. It has already been shown that thyroid improves the rate of hair growth in starved or underfed animals. When thyroid is given, the rate distribution of the underfed animals improves, when the comparisons are made on the basis of either the simultaneous controls, or the mean partial feeding rate. That is to say there are fewer below and more above the control or the mean rate.

		Acceleration	*Simultaneous partial feeding rate*	*Retardation*
IV.	Partial feeding +thyroid 0.2—1.3 gm	37 (7)	58 (11)	5 (1)
			Mean partial feeding rate	
V.	,,	58 (11)	37 (7)	5 (1)

It must be noted, that the simultaneous controls are from periods of partial feeding (without thyroid) and not normal feeding. If the hair growth rates (IV) are compared with the rates obtaining during normal feeding, the distribution is intermediate between that of normal feeding and partial feeding, viz.

		Acceleration	*Mean normal rate*	*Retardation*
VI.	Partial feeding +thyroid 0.2—1.3 gm	11 (2)	47 (9)	42 (8)

The point is made clearer when the mean rates alone are considered; under normal feeding the rate is 26 days, under partial feeding 41 days and under partial feeding *plus* thyroid, 30 days. Thyroid thus undoubtedly accelerates hair growth in a certain number of individuals but apparently does not entirely make good the nutritional deficiency.

The question arises, why does thyroid not accelerate the hair growth of all individuals, and what is the effect of varying quantities of the hormone? We shall first consider the effect of thyroid on normally fed animals.

		Acceleration	*Simultaneous normal feeding rate*	*Retardation*
VII.	Normal feeding +thyroid 0.2—1.3 gm	50 (9)	30 (7)	11 (2)

The above distribution, based on comparisons with the simultaneous control rates, shows that as far as individuals are concerned the majority are accelerated by thyroid. But as the mean hair growth rate of the controls of this series was somewhat higher than the mean for the entire

series of normal animals, comparison with the mean normal rate shows no acceleration, but on the contrary a higher percentage of retarded rates.

	Acceleration	*Mean normal rate*	*Retardation*
VIII. Normal feeding +thyroid 0.2—1.3 gm	0	72 (13)	28 (5)

The mean rate for this series of normally fed animals receiving thyroid was 27 days, which is actually one day longer than the mean for normal animals without thyroid (26 days).

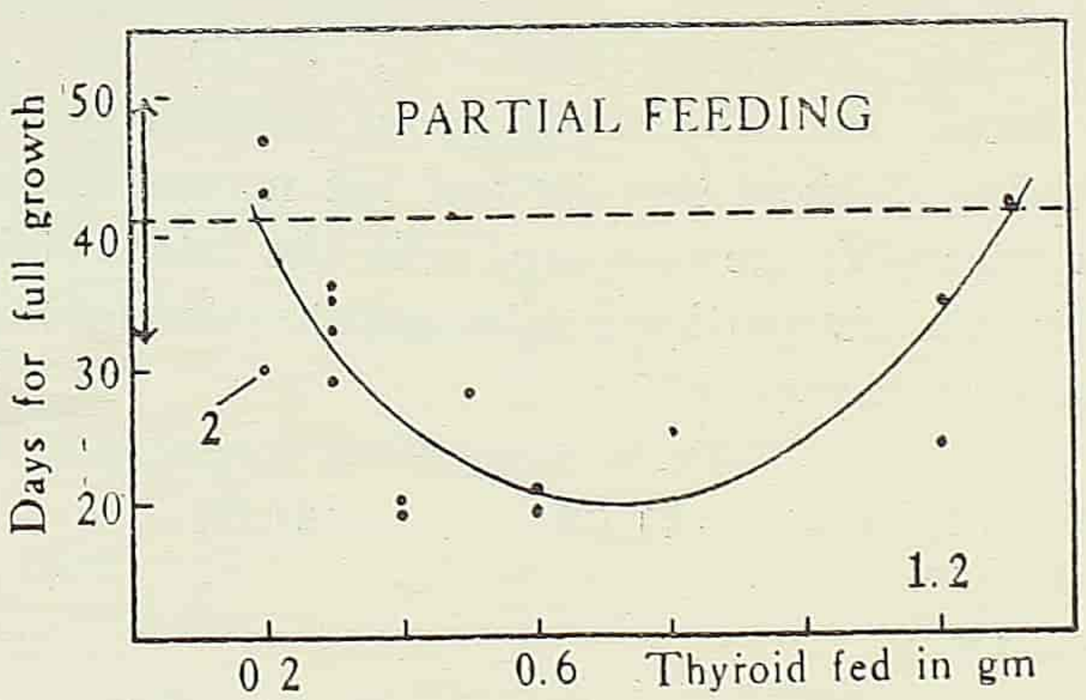

Fig. 2. Graph showing the influence of thyroid dosage on the rate of full hair growth in albino rats on a partial diet. Dotted line gives mean rate of hair growth in partially fed animals without thyroid; the arrows indicate the range $\pm 3E$.

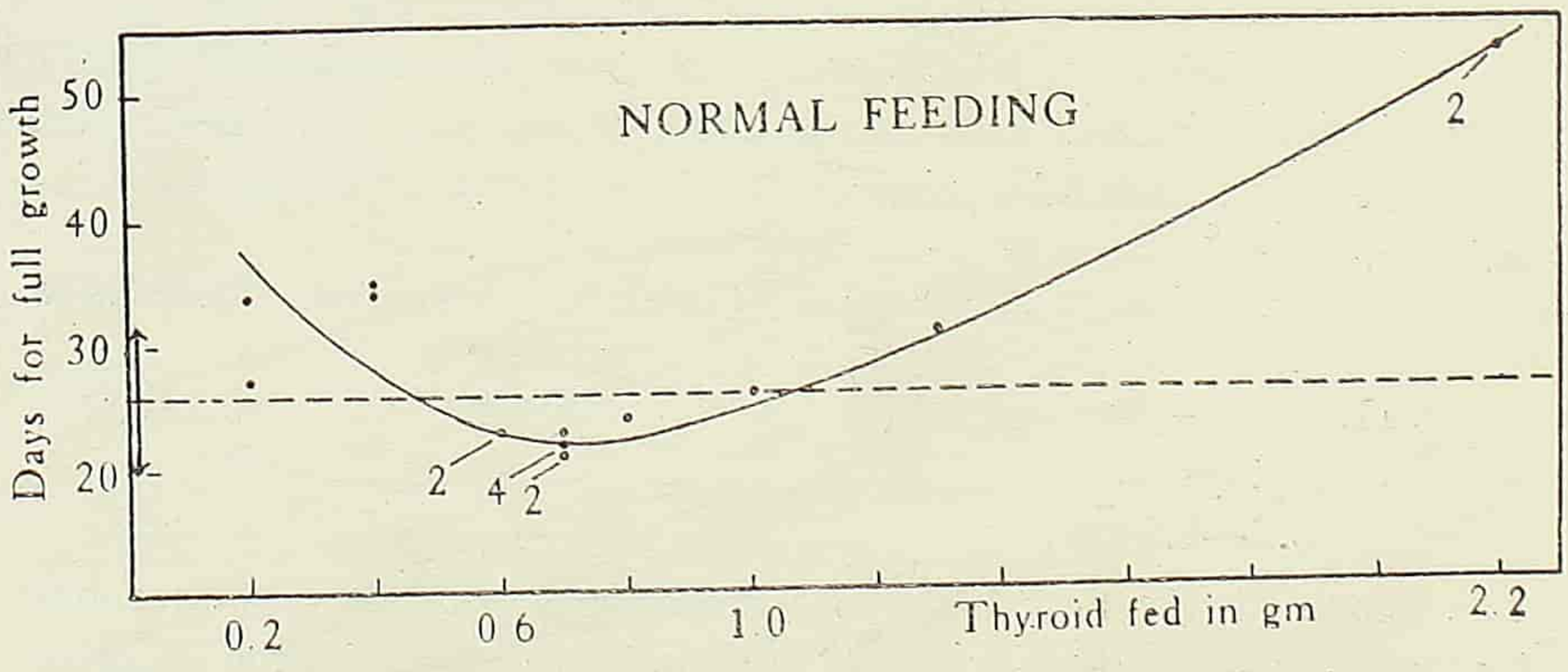

Fig. 3. Graph showing the influence of thyroid dosage on the rate of full hair growth in albino rats on a normal diet. Dotted line gives the mean rate of hair growth in normally fed animals without thyroid; the arrows indicate the range $\pm 3E$.

The result given above (VIII) appears to be in contradiction with the undoubted accelerating influence of thyroid in underfed animals. The effect of dosage, however, explains this apparent discrepancy. If figs. 2 and 3 are examined, it will be noted that the hair growth rates in both normal and underfed animals tend to be accelerated with dosages up to 0.6 or 0.7 gm thyroid. In the case of normally fed animals, the rates do not deviate beyond the "normal" range, whereas in underfed animals, the rate with 0.6 gm is definitely outside the range under this condition; it is in fact accelerated. With larger doses than 0.6 gm, the hair growth rate lengthens and with large doses (e.g., 2.2 gm per day) the growth of the hair is very definitely retarded. It is clear, therefore, that 0.6 –0.7 gm constitutes an optimal dosage, and that excess of thyroid brings about retardation instead of acceleration. Since we know that thyroidectomy retards hair growth, and that under-nutrition causes definite regression of the thyroid gland histologically (1, 2), it is clear in the light of our present experiments that the thyroid secretion is fundamentally responsible for the hair growth rate of the individual. In any group of normally fed individuals, we may expect varying levels of thyroid secretion, which may be classified as follows:

(i) Optimal — inducing hair growth at faster rates than the mean (viz., acceleration).

(ii) Slightly sub-optimal
or
(iii) Slightly supra-optimal
} — inducing hair growth at rates about the mean.

(iv) Markedly sub-optimal
or
(v) Markedly supra-optimal
} — inducing hair growth at rates beyond the mean (viz., retardation).

We regard the mean normal rate of hair growth as being just below optimal, and it is obvious that since over-dosage with thyroid depresses hair growth, that sub-optimal rates may be due either to insufficient thyroid secretion or to too much. Not only is the normal distribution of growth rates thus understandable, but also the effect of under-nutrition and thyroid feeding. The former will lower the level of thyroid secretion in all classes and thus lengthen the rate all round. While thyroid feeding will increase the level of thyroid in the body and thus improve (accelerate) the rates of individuals belonging to classes (ii) and (iv), it will depress those belonging to classes (i), (iii) and (v). Thus if the

animal is under-nourished the effect will be an acceleration tending to the optimal, i.e., the normal, while if the animal be already normal, the effect will tend away from the optimal towards depression or retardation.

TABLE 2.

The influence of thyroid on hair growth in normally and partially fed animals

Experiment	Animal No. and sex	Experimental condition	Age at start of experiment	Body weight		Days for complete hair growth after shaving
				Initial	Final	
			days	*gm*	*gm*	
C 13	1 ♂	Control	89	187	223	21
		,,	137	251	267	27
		,,	255	298	290	28
		,,	288	296	296	30
	2 ♂	Control	89	183	231	21
		2.2 gm thyroid	137	259	179	52+
		Thyroid discontinued		179	284	42
		Control	255	302	305	20
		0.71 gm thyroid	288	303	—	23
C 14	1 ♀	Control	112	133	139	25
		2.2 gm thyroid	141	133	108	52+
		Thyroid discontinued		108	158	39
	2 ♀	Control	112	143	152	21
		,,	141	153	173	36
C 17	1 ♀	Control	102	146	179	47
		Partial feeding +0.62 gm thyroid	188	131	134	20
		Partial feeding +0.59 gm thyroid	224	152	106	19
	2 ♀	Control	102	134	142	14
		Partial feeding	188	122	164	24
		,, ,,	224	166	144	32
P 9	1 ♂	Control	93	129	140	29
		Partial feeding	128	140	111	71+
	2 ♂	Control	93	124	150	27
		Partial feeding +0.5 gm thyroid	128	148	81	28

As we have already mentioned, thyroid feeding does not entirely compensate for lack of sufficient nutrition.

Examples of individual experiments are given in table 2.

Iodine. The dosage of iodine ranged from 0.2–1.2 gm. In more than half of the cases no change was produced, while the remainder exhibited acceleration and retardation of hair growth in about equal proportions. The results are compared with the individual hair growth rates during partial feeding, as the iodine experiments and those following were carried out under this condition, the object being to see if any of the substances investigated could replace thyroid.

Experiments	*Total dosage*	*Acceleration*		*No change*		*Retardation*	
	gm	*S.P.*	*M.N.*	*S.P.*	*M.N.*	*S.P.*	*M.N.*
IV. Partial feeding +thyroid	0.2–1.3	37 (7)	11 (2)	58 (11)	47 (9)	5 (1)	42 (8)
IX. Partial feeding +iodine	0.2–1.2	23 (4)	6 (1)	65 (11)	41 (7)	12 (2)	53 (9)
X. Partial feeding +tyrosine	0.1–0.2	20 (3)	7 (1)	55 (8)	33 (5)	27 (4)	60 (9)
XI. Partial feeding +tryptophane	0.1–0.3	36 (4)	0	18 (2)	18 (2)	46 (5)	82 (9)

S.P., comparison with partial feeding rate (simultaneous control).
M.N., comparison with mean normal rate.

Tyrosine. In 18 observations, there were four accelerations, 6 retardations and 8 without change. Two control observations made with the acid solvent (N/100 HCl) alone resulted in a slight acceleration of hair growth. In spite of this, there were more cases of retardation than acceleration.

Tryptophane. The largest number of retardations were recorded following tryptophane feeding.

While none of these three substances can equal the thyroid in their influence on hair growth, it is interesting to note that the effect of iodine approaches that of thyroid most closely and that tyrosine retards less than tryptophane. We would not lay undue stress on these observations except in so far that they show that thyroid cannot be replaced by any one of its constituents with the range of dosage given.

General remarks.

In some animals, hair falls easily when thyroid medication has been well on the way. This type of hair falling is different from that of infectious origin. Thus in parasitic infections, the hair falls out in patches resulting in areas of alopecia. The hair coat is on the whole dry and brittle. On the other hand, during thyroid feeding, the hair seems to become less firmly rooted so that if the hairs are lightly touched, a number will fall out. This occurs in spite of the fact that the coating is moist and glossy, looking "better" than that of the normal animal. At no time is general or patchy alopecia observed following thyroid feeding. These observations point to an endogenous factor. Probably the metabolism of the hair follicles is disturbed, perhaps accelerated, so that the follicles are in an unstable condition.

When the whole dorsum of the animal is shaved, hair grows in patches of different pattern whose distribution varies in different shavings. When some areas ("hair islands") have attained full growth, no hair may appear in the intermediary regions. This phenomenon has been observed in the rabbit by Schultz (4) and in mice by Collins (3). Even in the small shaved square, irregularity in hair growth sometimes happens especially at one or other corner. For no apparent reason, in one corner of the shaved square the hair refuses to grow. It may be that this "corner defect" falls in the area between the "hair islands". While poor regional blood supply may be the cause, the chance difference in the cyclic activity of the hair follicles, as pointed out by Trotter (5), may be another explanation. The latter is unlikely, however, as the chances of a defect occurring in the centre appears to be as good as the occurrence of a defect in the corner, yet a central defect is never observed. In the absence of any adequate explanation, all the readings complicated with the corner defect were discarded.

As noted before (2) and incidently mentioned above, the hair coating after thyroid medication becomes glosssy and soft. This happens frequently, and may or may not go together with acceleration of hair growth, indicating possibly a separate effect of thyroid. As it appears likely that the glossiness of the coat is related to the activity of the sebaceous glands, we are endeavouring to study the influence of thyroid on these structures.

SUMMARY

1. Partial feeding or starvation retards hair growth, but if thyroid is given in proper amounts, the hair may grow at nearly normal rates. Excess of thyroid depresses hair growth.

2. Since thyroidectomy also retards hair growth, while undernutrition causes regression of the thyroid gland, the thyroid secretion may be considered to be the most important factor controlling the growth of hair.

3. The optimal dose of thyroid is between 0.6 and 0.7 gm, larger doses depress hair growth.

4. It is suggested that the cause of the variation in hair growth rate in normal animals is variation in the individual level of thyroid secretion.

5. Iodine, tyrosine and tryptophane cannot replace thyroid.

6. Sex has no influence, but age has. The rate of hair growth is slower before 71 days than after, when it is relatively constant.

7. When thyroid is given the hair tends to become more glossy and to fall out more readily.

We are indebted to Dr. E. Tso for a supply of some of the experimental animals.

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甲狀腺乾粉，碘，色氨基酸，酥氨基酸，年齡及性屬對於毛髮生長之影響

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甲狀腺之有關於毛髮生長，前經下列試驗之結果以表明：(一)飼適量之甲狀腺乾粉，能使白鼠毛髮生長加速，(二)白鼠割去甲狀腺後，毛髮之生長較慢，(三)食物不足時，甲狀腺呈疲萎之象，毛髮之生長亦緩，(四)飼營養缺乏之白鼠以適量之甲狀腺乾粉，體重雖愈減而毛髮之生長則較速。

本篇研究之目的，爲進一步分析與甲狀腺精有關之各種成分，對於毛髮之生長有何影響，并視年齡及性屬與毛髮生長之關係，結果如下：—

甲狀腺乾粉如量適當，能助毛髮生長與先前試驗之結果相合。此適當之分量約在六百公絲上下。過此量則反有碍於毛髮之生長。

健康白鼠毛髮之生長率之不同，或由甲狀腺液多少之差別。

碘，色氨基酸，及酥氨基酸於髮之生長無確定之關係。

雌雄白鼠毛髮生長之速率無大分別。

未滿七十日之白鼠，毛髮生長較慢自七十日至二百六十日之間，年齡之差異與毛髮生長之速率無關係。

飼甲狀腺乾粉後，毛髮較常光澤而易脫掉。

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THE ALKALOIDS OF CORYDALIS AMBIGUA, CHAM ET SCH. (YEN-HU-SO) PART II. CORYDALIS F, G AND H.

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In a previous communication on Chinese Corydalis (Yen-hu-so) (1), the writer reported the isolation of five alkaloids from a non-phenolic fraction "2". They are provisionally named Corydalis A, Corydalis B, Corydalis C, Corydalis D, and Corydalis E, respectively. Corydalis A is identical with Corydaline in all respects and the other four are new. A preliminary examination of the physiological effects of Corydalis B by H.P. Chu (1) showed that it has narcotic and local anaesthetic properties and a cardiac augmentor action. The Corydalis B, used in Chu's experiments, was in the form of the hydrochloride which is only slightly soluble in cold water and causes some inconvenience in its administration. The preparation of other salts of Corydalis B was therefore attempted. It is now found that its neutral sulphate, crystallizing out from alcohol in six-sided prisms, is easily soluble in water, giving rise to a neutral solution, and consequently is more suitable for therapeutic use. The alkaloids, present in the other three fractions 1,3 and 4, have now been studied and 3 of them isolated in their pure state. Fraction 1, which is obtained by simply extracting the crude drug with benzene, consists of Corydalis A (Corydaline) and Corydalis D as its chief constituents. Fraction 3 which is phenolic and soluble in caustic alkalies, gives rise to two new phenolic bases, Corydalis F and Corydalis G, both of them having the same melting point but differing from each other in their optical activity, crystalline form and colour reactions. Corydalis F crystallises out from a mixture of chloroform and alcohol in the form of rhomboid crystals, melting at 237°C and has a specific rotation –250°.

Its molecular formula corresponds to $C_{20}H_{23}O_4N$ or $C_{20}H_{24}O_4N$. Corydalis G melts also at 237°, but crystallises out in the form of prisms and has a specific rotation of +300°. On account of the small amount of Corydalis G in hand, its molecular formula has not yet been determined. In 1923, Späth, Mosettig and Tröthandl (2) isolated from Corydalis tuberosa, corypalmine, a phenolic base, having a melting point 235–236°, a molecular formula $C_{20}H_{23}O_4N$ and a specific rotation (α) 16/D=+280°. It is consequently identical with Corydalis F in its melting point and molecular formula, but differs from it in its optical activity; whether it is identical with Corydalis G or not has to be further investigated. From fraction 4, a base in the form of its hydrobromide has been isolated and named Corydalis H. It is probably a quarternary base, as its hydrobromide is not decomposed by alkaline carbonates. When treated with barium hydroxide and extracted with chloroform, it gives rise to the formation of a new base which differs from Corydalis H by not giving the same hydrobromide. The other alkaloids present in Chinese Corydalis (Yen-hu-so) are still large in number and can only be fully investigated by working on a large quantity of raw material.

EXPERIMENTAL

(1) Sulphates of Corydalis B.

When Corydalis B was dissolved in a sufficient quantity of hot dilute sulphuric acid, on cooling its acid sulphate crystallised out as needles, m.p. 238°C. It was soluble with difficulty in cold water and strongly acid to litmus. Its neutral sulphate was obtained by treating Corydalis B with a calculated quantity of sulphuric acid in aqueous solution and evaporating the whole to dryness over a water bath. The residue was taken up with a little hot alcohol and set aside. The neutral sulphate crystallised out in the form of six-sided prisms, melting at 220°C. It was easily soluble in cold water, giving rise to a neutral solution.

(2) Corydalis F, $C_{20}H_{23}O_4N$ or $C_{20}H_{24}O_4N$.

It consists of the main constituent of fraction 3 which was separated from fraction 2 by means of a dilute solution of caustic soda. The phenolic bases were liberated from the sodium hydroxide solution by treating it with ammonium chloride and extracting with chloroform. The chloroform solution, when dried and distilled, left behind a crude phenolic basic residue. The whole was converted into its hydrobromide

in aqueous solution. When crystallised pure, the hydrobromide was worked up for Corydalis F by dissolving in water, making alkaline with potassium carbonate and extracting with chloroform. The crude Corydalis F so obtained was further purified by repeatedly recrystallising from chloroform with the addition of alochol until a constant melting point was obtained. Corydalis F forms colourless rhomboid crystals (see fig. 1) m.p. 237°C. It was sparingly soluble in organic solvents except chloroform, in which it was easily soluble on warming. Its chloroform solution and also its crystals became coloured on keeping. It dissolved in a dilute solution of caustic alkali forming a colourless solution. A 0.4 per cent solution in chloroform gave a specific rotation –1°, in 1 dm tube, (α) 25/D = –250°. Its molecular formula has been found to be $C_{20}H_{23}O\ N$ or $C_{20}H_{24}O_4N$ according to the following analysis:—

(i) 0.1180 gm substance gave 0.3036 gm CO_2 and 0.0757 gm H_2O,
C=70.16; H=7.12.

(ii) 0.1180 gm substance gave 0.3034 gm CO_2 and 0.0735 gm H_2O.
C=70.12; H=6.92.

(iii) 0.1194 gm substance gave 4.40 cc moist nitrogen at 25°C and 755 mm pressure, N=4.06.

Calculated for the formula	$C_{20}H_{23}O_4N$	or	$C_{20}H_{24}O_4N$
	C=70.38		C=70.17
	H = 6.74		H = 7.01
	H = 4.10		N = 4.09

This substance may therefore have one of the above two formulae.

It showed the following colour reactions; concentrated sulphuric acid, colourless; Erdmann's reagent, yellowish orange; Froehde's reagent, violet and then blue. Its hydrobromide and hydrochloride were prepared.

(a) *Hydrobromide.* Prepared by dissolving pure Corydalis F in a slight excess of hot dilute hydrobromic acid. It separated out as a jelly at first, but was crystallised by warming the liquid. It formed prismatic needles (see fig. 2), melting at 222°C to a reddish liquid. It was soluble in water and alcohol.

(b) *Hydrochloride.* Obtained by dissolving Corydalis F in an excess of dilute hydrochloric acid. In pure water and alcohol, it was very soluble. It formed fine needles and contained some water of crystallisa-

tion. When air-dried, it melted below 100°C to a clear colourless liquid, then solidified gradually as the temperature rose and finally melted at 234°C.

(3) Corydalis G.

During the process of purifying Corydalis F by means of its hydrobromide, there was found a hard crust sticking to the bottom of the flask. It was separated, purified by recrystallising in water and converted into its free base as usual. The phenolic base so obtained, after being recrystallised several times from a mixture of chloroform and alcohol, formed prisms, (see fig. 3) melting constantly at 237°C, readily soluble in hot chloroform, but not in other organic solvents in which it was only sparingly soluble. Unlike Corydalis F it was dextro-rotatory; a 0.4 per cent solution in chloroform gave a specific rotation +1.20° in 1 dm tube, (α) 25/D = +300°. It showed the following colour reactions; concentrated sulphuric acid, colourless; Erdmann's reagent, yellowish orange; Froehde's reagent, violet, green and then blue. The last colour reaction differentiates Corydalis G from Corydalis F. Its hydrobromide crystallised out from water as prisms (see fig. 4), m.p. 247°C. On account of the small quantity of Corydalis G isolated in a pure state, the molecular formula has not been determined. An intimate mixture of Corydalis F and Corydalis G melted at 214°C.

(4) Corydalis H.

The fraction 4, an alcoholic extract as described in a previous communication (1) was concentrated in vacuo at low temperature. The aqueous residue which contained very little alcohol was first extracted with benzene to remove impurities, (and also some basic substances escaped from previous benzene extractions) and then with chloroform. The chloroform extract, when dried and distilled, left behind a dark resinous residue which was taken up with a little alcohol and allowed to crystallise. After standing in a cool place for several months, no crystals could be isolated. Acidified with hydrobromic acid, well formed prisms were obtained. When crystallised pure from alcohol, it formed yellowish long prisms (see fig. 5), m.p. 235°. It was found to be a hydrobromide and named Corydalis H. It was soluble in water, alcohol and chloroform. Its aqueous solution was optically inactive (α) 20/D=0°. A further quantity of the same hydrobromide was obtained from the above aqueous residue by acidifying with hydrobromic acid and extracting with chloroform. When dissolved in water, made alkaline with

potassium carbonate and extracted with chloroform, only unchanged hydrobromide was recovered. With excess of potassium carbonate until saturation, the substance partially resinifies. When treated with barium hydroxide and extracted with chloroform, a new base was obtained. It crystallised out from alcohol in the form of groups of plates (see fig. 6), melting at 153°C. It was insoluble in water, and soluble in alcohol and chloroform. Its hydrobromide salt melted at 228° instead of 235°, the melting point of the hydrobromide of Corydalis H.

SUMMARY

1. From the tubers of Chinese Corydalis (Yen-hu-so), Corydalis Ambigua, Cham et Sch. in addition to the five alkaloids already described in the last communication (1) three more new alkaloids have now been isolated and named Corydalis F, Corydalis G and Corydalis H. The first two are phenolic and the third is quarternary.

(a) Corydalis F.

$C_{20}H_{23}O_4N$ or $C_{20}H_{24}O_4N$ rhomboid crystals, m.p. 237°;

(α) 25/D = −25.5°.

Hydrobromide; prismatic needles, m.p. 222°.

Hydrochloride, fine needles, melts in its water of crystallisation below 100°C, then solidifies and finally melts at 234°C.

(b) Corydalis G, prisms, m.p. 237°; (α) 25/D = +300°; hydrobromide prisms, m.p. 247°.

(c) Corydalis H, obtained only in the form of its hydrobromide, m.p. 235°C; (α) D = 0°. When treated with barium hydroxide and chloroform, a new base was derived, melting at 153°C.

2. Two sulphates of Corydalis B have been prepared, one acid and the other neutral, the latter is easily soluble in cold water and suitable for therapeutic use.

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STUDIES ON DENATURATION OF PROTEINS

IX. LIBERATION OF NON-PROTEIN SUBSTANCES UPON DENATURATION AND COAGULATION OF PROTEINS

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Wu and Yen (6) showed that some non-protein substance capable of reacting with the phenol reagent of Folin and Denis was liberated when egg albumin was denatured by alkalies and, to a much smaller extent, by acids. Wu and Wu (5) showed that the liberation of non-protein chromogenic substances also accompanied heat denaturation of egg albumin. Mastin and Rees (2) repeated our work on heat denaturation but failed to confirm our finding.

Since the liberation of non-protein substances occurs in denaturation of egg albumin by alkalies much more readily than in denaturation by acids, it seemed to us that the reaction at which the protein is denatured by heating might be an important factor and that it might offer an explanation for the discrepancy between their results and ours.

In our previous study the egg albumin was recrystallized two or three times and possibly contained some ovomucoid. This was thought to be immaterial, since it was believed that the ovomucoid would be left in the filtrate from the control as well as in that from the heated albumin solutions. Subsequent experiments have shown that it is important to use an albumin preparation which is free from ovomucoid. Ovomucoid, when present alone, is not precipitated by tungstic acid. However, when tungstic acid is added to a solution of natural egg albumin containing ovomucoid, this is adsorbed by the precipitated albumin. Denatured and flocculated albumin behaves similarly, but coagulated albumin adsorbs little or no ovomucoid in the presence of tungstic acid. Hence if the albumin preparation contains some ovomucoid, the filtrates prepared from natural, denatured, and coagulated

albumins by means of tungstic acid would contain different amounts of ovomucoid and would give an erroneous picture of the amount of non-protein chromogenic substances liberated from the egg albumin. In the present study the egg albumin preparation used was highly purified, and probably free from ovomucoid but to avoid the possibility of differential adsorption of this substance tungstic acid was not used for the preparation of the non-protein filtrates from the heated albumin solutions.

Egg albumin was recrystallized six times and dialyzed in the presence of toluene in collodion tubes and chloroform in the water outside. An approximately 0.5 per cent solution was prepared and to 40 cc portions of this solution graded amounts of N/10 HCl or NaOH were added. Two sets of such solutions were prepared. One set was used for pH determinations and the other set was plunged into a bath of boiling water and allowed to remain there for 15 minutes. After cooling, the solutions were neutralized with sodium carbonate or acetic acid and proper amounts of water were added to equalize the volume (48 cc). The protein that was thus coagulated or denatured and flocculated was removed by filtration. The filtrates were heated once more in boiling water for 10 minutes.

The purpose of the second heating is to remove any trace of conalbumin which might be present in the albumin preparation. Conalbumin is not denatured by heating in slightly acid solution, but it can be coagulated by heat at its isoelectric point. It may be reasonably assumed that the amount of non-protein chromogenic substances liberated from the trace of conalbumin by this second heating is quantitatively negligible.

Any coagulum that might be formed on the second heating was removed by filtration. A non-protein filtrate from unheated albumin was prepared by adding to 40 cc of the albumin solution 2 cc H_2O and 3 cc each of 10 per cent sodium tungstate and 2/3 N sulphuric acid. For the determination of non-protein chromogenic substances, to 25 cc portions of the filtrates were added 0.5 cc of phenol reagent and 3 cc of 20 per cent Na_2CO_3. Tyrosine solutions were used as standards. The results are shown in table 1 and fig. 1.

If any ovomucoid were present in the albumin solution, it would be carried down by the tungstic acid precipitate in the control and not by the heat coagulum. The filtrate from the unheated control would contain less total chromogenic substances than that from the albumin

TABLE 1.

Liberation of non-protein chromogenic substances from egg albumin

Mg "tyrosine" per 100 mg albumin

pH	Chromogenic substances	pH	Chromogenic substances
1.4	0.179	6.3	0.125
1.9	0.164	6.7	0.124
3.2	0.123	7.2	0.123
4.0	0.132	8.0	0.164
4.3	0.125	8.3	0.180
4.5	0.123	9.4	0.191
5.1	0.105	10.3	0.225
5.5	0.112	11.3	0.293
5.8	0.110	Control	0.103

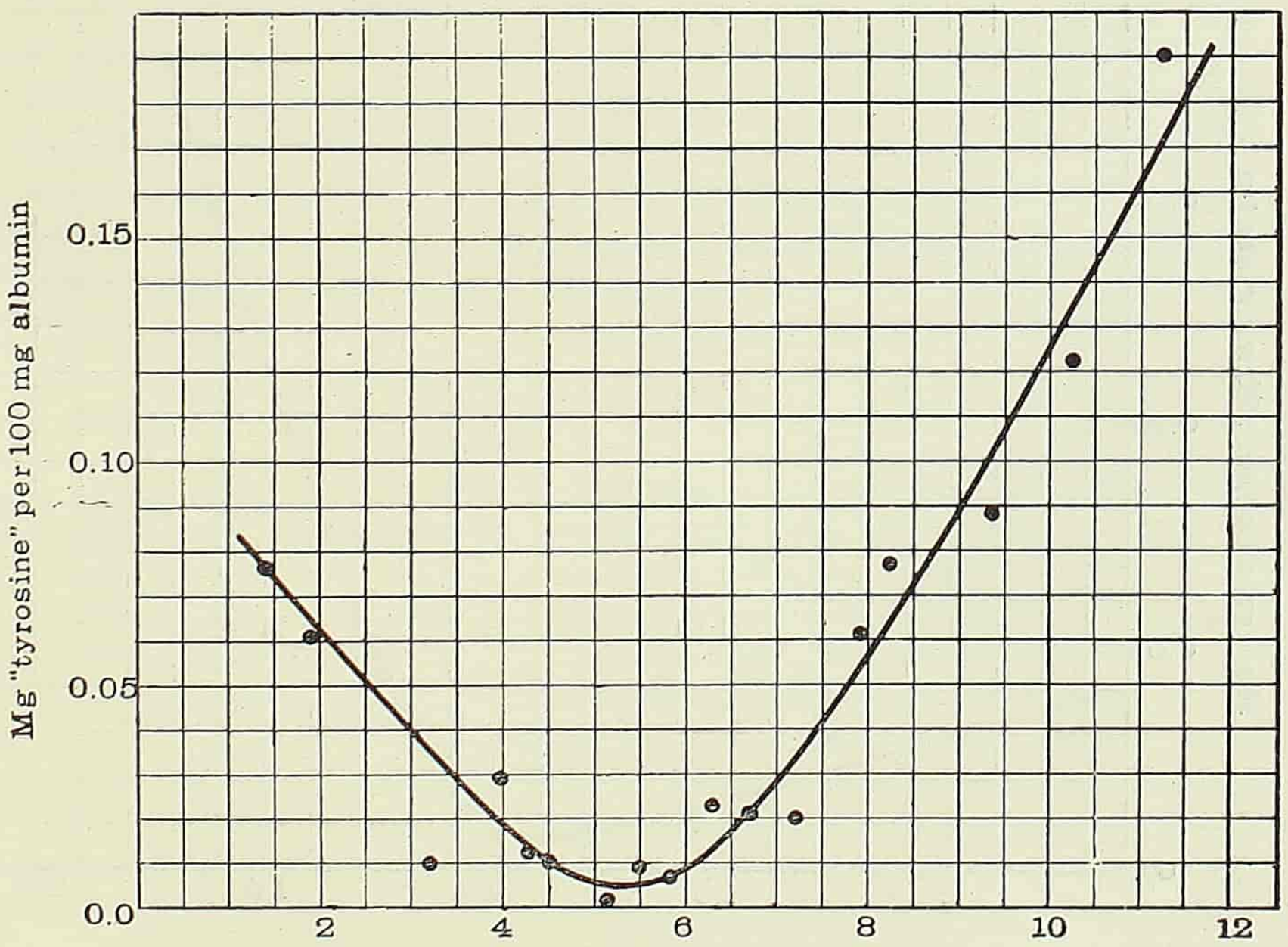

Fig. 1. Liberation of non-protein chromogenic substances on denaturation and coagulation of egg albumin.

solution coagulated at the isoelectric point. From table 1 it will be noted that the two were practically the same. We may conclude, therefore, that the albumin solution used was free from ovomucoid and that the liberation of non-protein chromogenic substances observed in the alkaline and acid solutions was real and not due to differential adsorption of that substance.

At the isoelectric point there is practically no liberation of non-protein chromogenic substances. On either side of this point there is some liberation, much more on the alkaline than on the acid side. Since it has been shown that coagulation is not denaturation followed by flocculation but an entirely different process (3) and that heating of egg albumin at the isoelectric point probably causes coagulation without denaturation (4), we may conclude that coagulation of egg albumin is not, but denaturation is, accompanied by liberation of non-protein chromogenic substances.

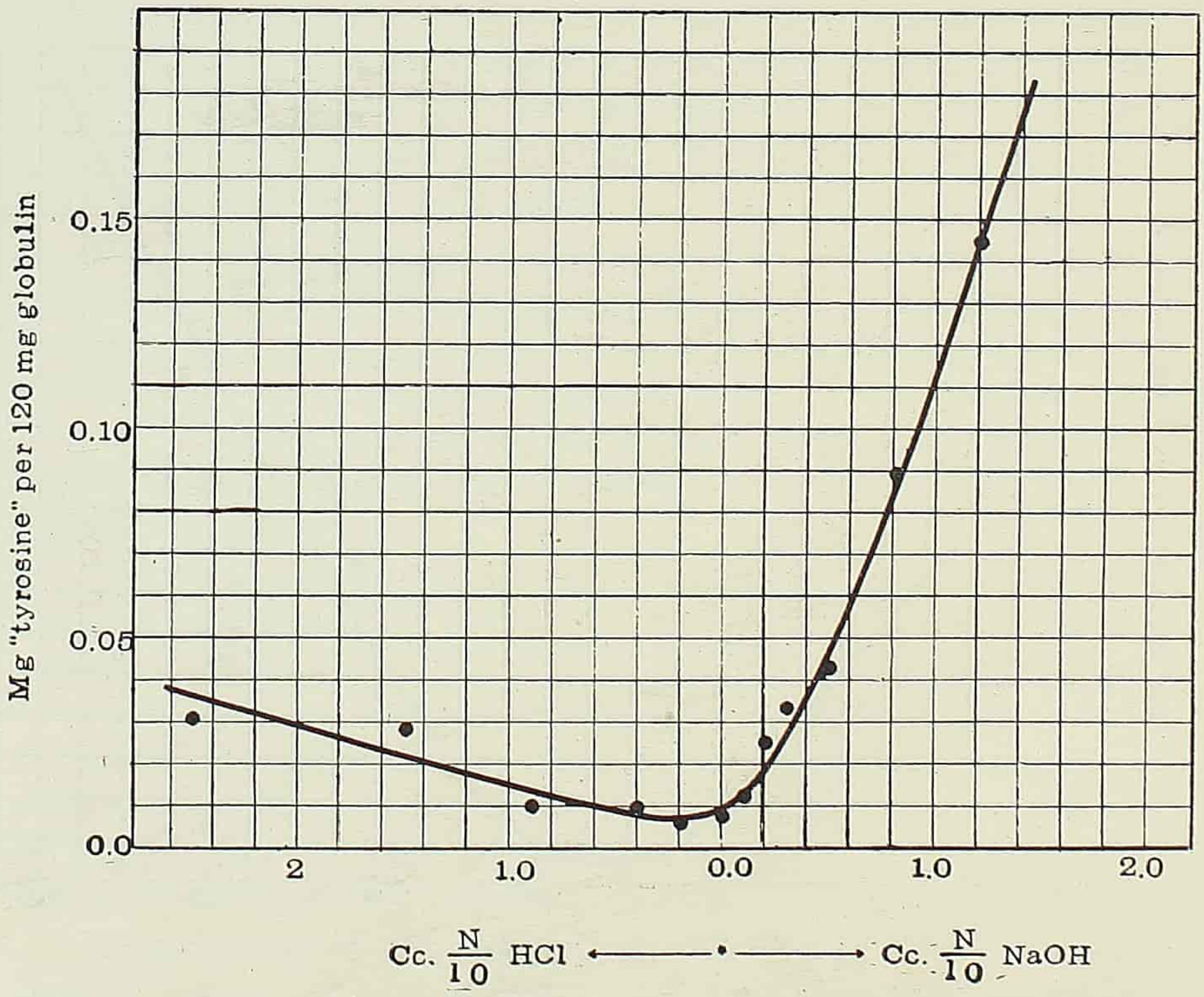

Fig. 2. Liberation of non-protein chromogenic substances on denaturation and coagulation of sheep's serum globulin.

We have studied also sheep's serum globulin and hemoglobin by methods similar to that described above, excepting that tungstic acid was used for the removal of the protein. The former behaves like egg albumin (fig. 2), but the latter shows no detectable liberation of non-protein chromogenic substances. It would seem, therefore, that while the liberation of non-protein chromogenic substances may be a necessary result of denaturation of some proteins, it is not so for all proteins.

In our study on denaturation of egg albumin by dilute acids and alkalies, we observed that the course of liberation of the non-protein substances did not run parallel with that of denaturation and we concluded that the liberation of these non-protein substances was not an essential feature of the denaturation of proteins (6). In our paper on heat denaturation of egg albumin we mentioned the liberation of non-protein chromogenic substances to show the similarility of denaturation of this protein by heat and that by acids and alkalies in the cold, and we did not take it as an observation upon which a general theory of denaturation could be based. Lloyd (1) attributes to us the view that the liberation of non-protein chromogenic substances is an evidence of the hydrolytic nature of denaturation. While we are of the opinion that denaturation is a mild hydrolysis or some other kind of degradation our view is not based on that observation.

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蛋白質之變性作用

九。 蛋白質變性或凝固時非蛋白質化合物之解放

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鷄蛋白質於加熱變性時，因分子一部之變化，而解放某種非蛋白質之化合物；於加熱凝固時則不然。加熱變性時之酸度或鹼度愈高，則所解放之量亦愈多。

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COMPARATIVE STUDIES OF EPHEDRINE, RACEMIC EPHEDRINE AND PSEUDOEPHEDRINE

II. COMPARATIVE TOXICITY

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In our previous communication (10) we showed qualitative and quantitative differences in the action of ephedrine, ephetonin and pseudoephedrine. Curtis (5) in showing that ephedrine is more active than ephetonin found that the difference was not so pronounced for minute doses of these drugs. Whereas 0.05 mg per kilogram body-weight in cats showed little difference, 4 mg produced very divergent effects. He explains Kreitmair's (7) original claim for identical quantitative effects by the small dosage used. Berger (2) in summarizing extensive clinical studies upon ephedrine and ephetonin considered ephetonin less toxic than ephedrine stating that in cases of heightened sympathetic irritability the possible by-effects, "are incomparably more frequent and more pronounced with ephedrine than with ephetonin". Certain by-effects related to the neurotropic action of ephedrine can be so considered, but in view of the greater musculotropic effect of ephetonin it is quite logical that Berger should have observed with ephetonin cardiac palpitations and untoward effects of muscular origin. This and other general evidence shows that there is a qualitative difference in action, a quantitative difference in effect, and differences in the minor toxic effects of these two isomers. There remains the question of absolute and relative toxicity from the lethal standpoint.

A study of the comparative action and toxicity of ephedrine and pseudoephedrine has been made by Fujii (6). He concluded that for the frog pseudoephedrine was less toxic in the proportion of 1 to 1.6, but for the mouse ephedrine had a larger lethal dose in the proportion of 1 to 0.8 toxicity for pseudoephedrine and ephedrine respectively.

These results leave the question of the relative toxicity of these two substances in a somewhat indefinite position. So we have taken a much larger variety of animals, frogs, hamsters, rats, gray and white rabbits, and dogs to determine this point, at the same time we have made a comparison with racemic ephedrine, referred to as ephetonin, and we have made a careful study of the toxicity of pseudoephedrine.

A comparison of the toxicity of ephedrine and ephetonin was made by Kreitmair (7). He used white mice and obtained identical results. Chen (3) confirmed this conclusion by a series of rabbit experiments in which he obtained a minimum lethal dose for both isomers of 60 mg per kilogram by intravenous injection. We have not repeated Kreitmair's work because Fujii's results show how differently from other animals mice react to the ephedrines. Our results with hamsters, a kind of field mice, are similar in showing a relative effect different from any of the other animals used. In white rabbits Chen's minimum lethal dose (3,4) for ephedrine does not agree with that reported by Nagel (9), or Kreitmair (7), both of whom gave 50 mg.

The minimum lethal dose of ephedrine has been reported upon by various workers. Miura (8) in the original work upon its toxicty used frogs, rabbits (subcutaneously), and dogs (subcutaneously), and gave his results as 400 to 500, 300 to 460, and 220 mg respectively. Amatsu and Kubota (1) obtained the same result with frogs, Fujii found the higher figure to be true for his animals, which may have been a different species of Rana. Kreitmair (7) and Chen (4) both reported larger values, which we are inclined to believe are due to salts not entirely free from isomers. It is well known that different species have been used. In conducting our experiments we at first found considerable variation in result, which was later corrected by improved technique. These points emphasize the need for results obtained from identical species of animals, the same purity of the isomers, and similar technique. Fair comparative values are hard to make by comparing results obtained in different laboratories, with varying species of animals, and by a technique that may be not quite the same, e.g., there is too big a difference in the cat intravenously as observed by Kreitmair (7) and Chen (4), 60 and 75 mg respectively, for a good comparison to be made with these figures and that possibly obtained by a third worker upon ephetonin or pseudoephedrine.

Most work has been done with white rabbits. Fujii's (6) experiments confirmed Miura's (8) earlier results obtained by subcutaneous

injection, and Chen (4) brought the figures within the narrower limits of 320 to 400 mg. He also administered the drug orally and intraperitoneally. We have only repeated the series by intravenous injection which for the sulphate Chen gave as high a figure as 66 to 70 mg.

EXPERIMENTAL

I. Administration of the drugs.

These experiments have extended over two years, during which time season and temperature have shown a marked influence upon the results obtained. This was especially so in the case of the hamster, which may show 100 per cent difference in the minimum lethal dose in midsummer and autumn. The doses of ephedrine definitely fatal when summer heat was between 35° and 38°C, were well tolerated in early autumn when the temperature dropped to 24° to 27°. The usual technique was followed for subcutaneous, intravenous and intraperitoneal injection, care being taken to use solutions of the isomers of similar concentration, for we observe differences in our results when the strengths of the solutions have too great a variation.

In frogs the drug was injected through the mouth into the anterior lymph sac. At first it was found difficult to obtain consistent results. Using male frogs only and squeezing out the urine from the bladder, we were able to obtain the desired consistency.

TABLE 1.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in dogs by intravenous injection of 10 per cent solutions

Ephedrine HCl			Ephetonin HCl			Pseudoephedrine HCl		
Body weight and sex of dogs	Dose per kg	Results	Body weight and sex of dogs	Dose per kg	Results	Body weight and sex of dogs	Dose per kg	Results
	mg			*mg*			*mg*	
7.05 kg, ♀	50	Recovered	3.88 kg, ♀	90	Recovered	5.55 kg, ♀	100	Recovered
8.4 kg, ♂	60	Dead	5.38 kg, ♀	90	Recovered	6.95 kg, ♀	100	Recovered
8.1 kg, ♀	60	Recovered	5.59 kg, ♀	90	Dead	4.15 kg, ♀	110	Recovered
4.6 kg, ♀	60	Recovered	4.00 kg, ♂	100	Recovered	6.91 kg, ♀	110	Dead
7.75 kg, ♀	70	Dead	9.43 kg, ♀	100	Dead	3.67 kg, ♂	120	Recovered
5.7 kg, ♀	**70**	*Dead*	13.77 kg, ♂	**100**	*Dead*	9.34 kg, ♂	120	Recovered
			5.05 kg, ♀	110	Dead	7.55 kg, ♀	120	Dead
			5.78 kg, ♀	110	Dead	5.53 kg, ♀	130	Recovered
						3.56 kg, ♀	130	Dead
						6.96 kg, ♀	**130**	*Dead*

July 1928. Temperature 29°to 32°C.

II. The minimum lethal dose.

1. *Dogs.*—Using the hydrochloride salts we repeated the work of Chen (4) upon ephedrine sulphate and made further tests of the lethal dose of ephetonin and pseudoephedrine. The results in table 1 confirm Chen's figure (4) for ephedrine and show that ephetonin is only 70 per cent as toxic and pseudoephedrine is 54 per cent.

TABLE 2.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in white rabbits by intravenous injection of 5 per cent solutions

Ephedrine HCl			Ephetonin HCl			Pseudoephedrine HCl		
Dose per kg	Rabbits used	Rabbits died	Dose per kg	Rabbits used	Rabbits died	Dose per kg	Rabbits used	Rabbits died
mg			*mg*			*mg*		
40	2	0	58	1	0	65–90	5	0
50	2	2	60	3	1	100–11	10	3
60	1	1	**70**	3	2	120	3	1
						130	4	3
			80	3	2	135	3	3
70	1	1				140	2	2

July 1928. Temperature 29° to 30°C.

2. *Rabbits.*—The M.L.D. in white rabbits by intravenous injection did not show identical results for ephedrine and ephetonin as reported by Chen (3). Pseudoephedrine is least toxic to the white rabbit, and by this path of administration shows a greater difference from ephedrine than by any other (see table 2). On the other hand for gray rabbits (see table 3) pseudoephedrine is highly toxic while ephetonin is more toxic to white rabbits than to gray rabbits by intravenous injection (see table 4). Subcutaneously in the gray rabbit the relative effects of the three isomers is similar to intravenous injection in the dog (see table 4). In all cases both in dogs and rabbits ephedrine, ephetonin and pseudoephedrine show different degrees of strength similar in proportion to our results from these animals in blood pressure experiments previously reported.

3. *White rats.*—Excluding our results with the hamster which are discussed separately, ephedrine and ephetonin showed the closest similarity of results of any, by subcutaneous injection in the white rat, see table 5. Chen (4) found the M.L.D. in the rat by intravenous injection quite large, nearly twice as great as in other animals like the dog and

TABLE 3.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in gray rabbits by intravenous injection

Dose per kg of body weight	Ephedrine HCl		Ephetonin HCl		Pseudoephedrine HCl	
	Rabbits used	Rabbits died	Rabbits used	Rabbits died	Rabbits used	Rabbits died
mg						
60 to 75	8	0				
80	**3**	**2**	2	0	1	0
85	1	1	3	1		
90			**2**	**2**	3	1
100			1	1	**2**	**2**
110 to 130			1	1	3	3

June 1928. Temperature 27.5° to 31°C.

TABLE 4.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in gray rabbits by subcutaneous injection

Ephedrine HCl			Ephetonin HCl			Pseudoephedrine HCl		
Dose per kg	Rabbits used	Rabbits died	Dose per kg	Rabbits used	Rabbits died	Dose per kg	Rabbits used	Rabbits died
mg			*mg*			*mg*		
180–210	7	1	300–340	11	1	380	1	0
220	1	0	350	2	0	390	2	0
230	3	2	**360**	3	2	**400**	2	2
240	2	2	370	2	2	430	1	1
250	2	2				470	1	1
260	2	2				500	1	1
270	2	1				530	1	1
280–420	8	8						

July 1928. Temperature 27.5° to 32.5°C.

the cat. Subcutaneously the rat and the gray rabbit do not show such a marked difference the M.L.D. being 320 and 230 mg respectively. With ephetonin this was still more the case, there being very little difference shown in these two animals, see table 9, in which is summarized the various results obtained.

TABLE 5.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in rats by subcutaneous injection

Ephedrine HCl			Ephetonin HCl			Pseudoephedrine HCl		
Dose per kg	Rats used	Rats died	Dose per kg	Rats used	Rats died	Dose per kg	Rats used	Rats died
mg			*mg*			*mg*		
290	2	0	300	1	0	350	1	0
300	3	0	310	1	0	380	1	0
310	5	2	330	1	0	450–660	7	0
320	**5**	**4**	340	2	0	670	3	1
330	2	2	**350**	**2**	**2**	**680**	**3**	**3**
350	1	1	400	1	1			
370	1	1						

September 1928. Temperature 23° to 25°C, 5 per cent solution, rats weighing 230 to 484 gm.

4. *Frogs.*—In table 6 is reported our results obtained with male frogs. We were able to secure more clear cut results by using the one sex, by purifying our alkaloids, and by taking care that the bladders of the animals were emptied before taking the weight.

TABLE 6.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in male frogs by injection into the anterior lymph sac

Ephedrine HCl			Ephetonin HCl			Pseudoephedrine HCl		
Dose per kg	Frogs used	Frogs died	Dose per kg	Frogs used	Frogs died	Dose per kg	Frogs used	Frogs died
mg			*mg*			*mg*		
500	6	2	580	6	0	730	6	0
510	6	2	590	6	1	740	6	0
520	6	2	600	12	3	750	6	0
530	12	3	610	12	3	760	12	2
540	12	8	620	12	5	**770**	12	**7**
550	12	7	**630**	6	4	780	6	4
560	6	5	640	6	3	790	12	6
570	6	4	650	6	4	800	6	**3**
580	6	4	660	6	4	810 to		
590	6	5	670	6	5	850	42	25
600	6	6	680	6	6			
610	6	6	690	6	5			
620	6	6	700	6	6			

June 1928. Temperature 27.5° to 31°C.

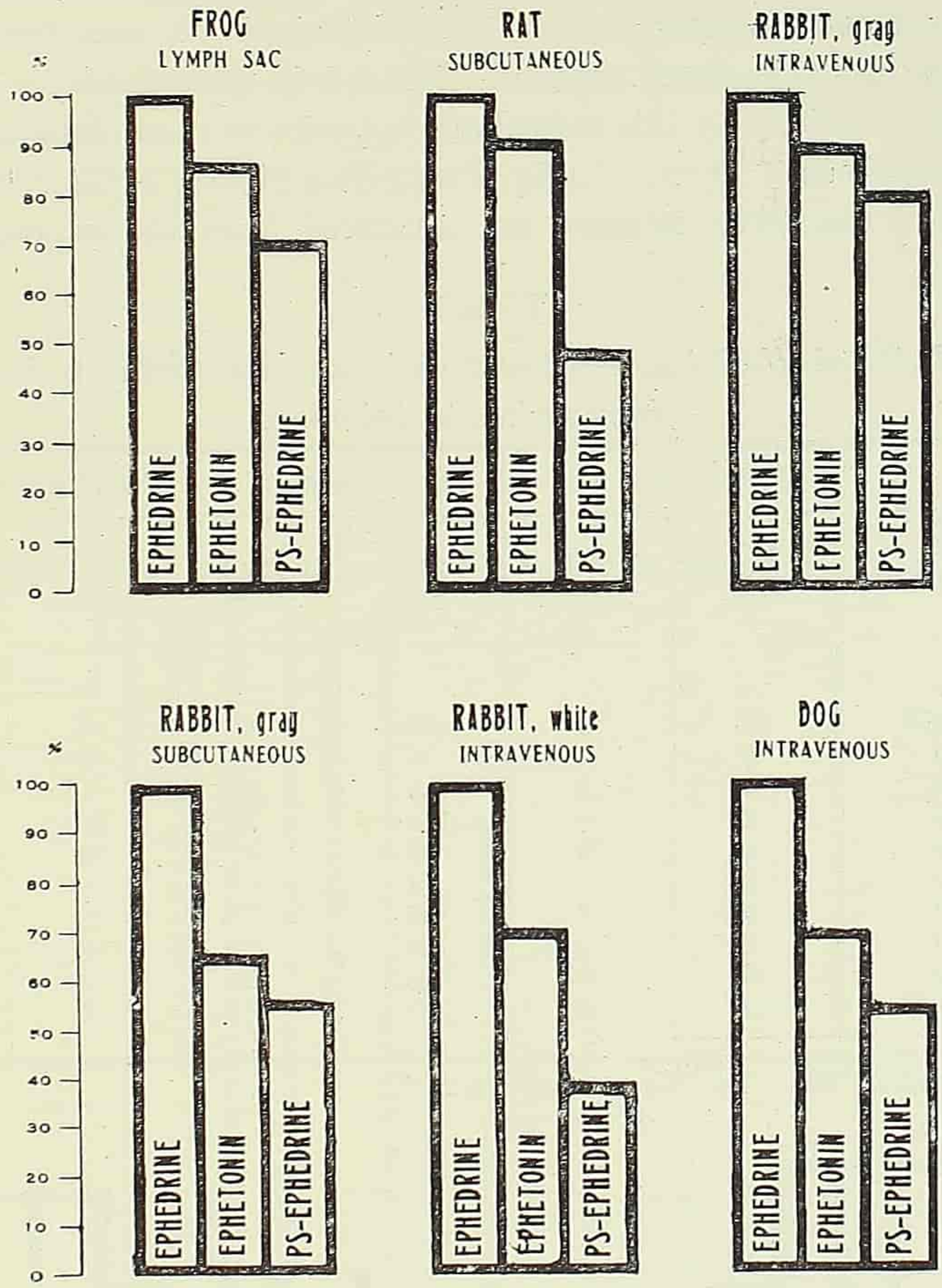

Fig. 1. Comparative magnitudes of M.L.D. of ephedrine, ephetonin and pseudoephedrine in different animals by different methods of administration.

It should also be noted that our work was done in June at a temperature of 27.5 to 31°C. A short series performed in the late autumn gave a slightly lower figure for ephedrine but it required fifteen per cent more of pseudoephedrine to kill the animals. Another series was conducted in very hot, damp summer weather. In all cases the lethal dose was smaller by eight to nineteen per cent 500, 590 and 650 mg for ephedrine, ephetonin and pseudoephedrine as compared with 540,630 and 770 mg required under more normal conditions. It seems as if temperature had an influence, especially upon pseudoephedrine which is more musculotropic than ephedrine.

5. *Hamsters.*—Although intraperitoneal injection is liable to show irregular results on account of poor technique, it was the simplest procedure for this small animal. Table 7 is remarkable in that the comparative effects in this animal are opposite to those found in all the other animals used by us. In fig. 2 we give a picture of the comparative toxicity of the three isomers, as calculated from the average results

TABLE 7.

The M.L.D. of ephedrine, ephetonin and pseudoephedrine in hamsters by intraperitoneal injection

Dose per kg of body weight	Ephedrine HCl		Ephetonin HCl		Pseudoephedrine HCl	
	Hamsters used	Hamsters died	Hamsters used	Hamsters died	Hamsters used	Hamsters died
mg						
200–270	12	3	12	2	6	1
280	3	0	6	2	0	0
290	0	0	3	0	0	0
300	6	0	15	7	6	2
310	9	3	**9**	**7**	**9**	**7**
320	9	2	9	6	9	6
330	9	3	6	6	9	7
340	6	0	3	2	3	3
350	**6**	**4**	3	2	3	3
360–400	12	12	0	0	6	6

September 1928. Temperature 20° to 27°C.

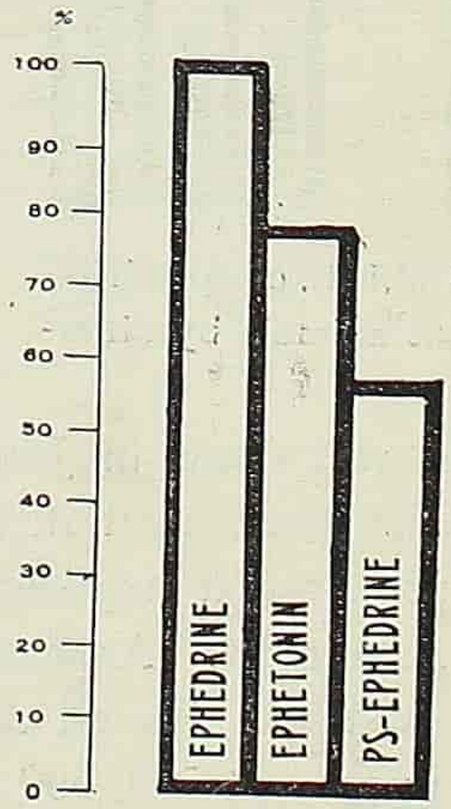

Fig. 2a. Comparative magnitudes of average M. L. D. of ephedrine, ephetonin and pseudoephedrine from various animals.

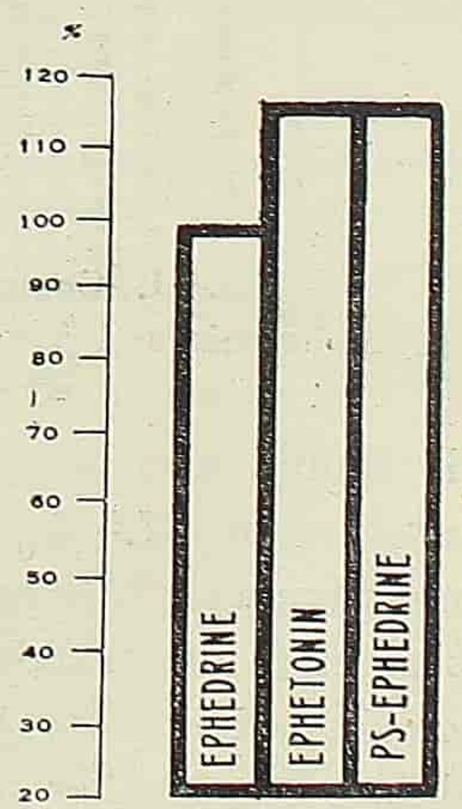

Fig. 2b. Comparative magnitudes of M. L. D. of ephedrine, ephetonin and pseudoephedrine in hamster by intraperitoneal injection.

obtained with frogs, dogs, rats, white and gray rabbits, summarized in table 9. Figure 2 shows how different a picture is obtained with hamsters, in which ephetonin and pseudoephedrine are both more toxic than ephedrine.

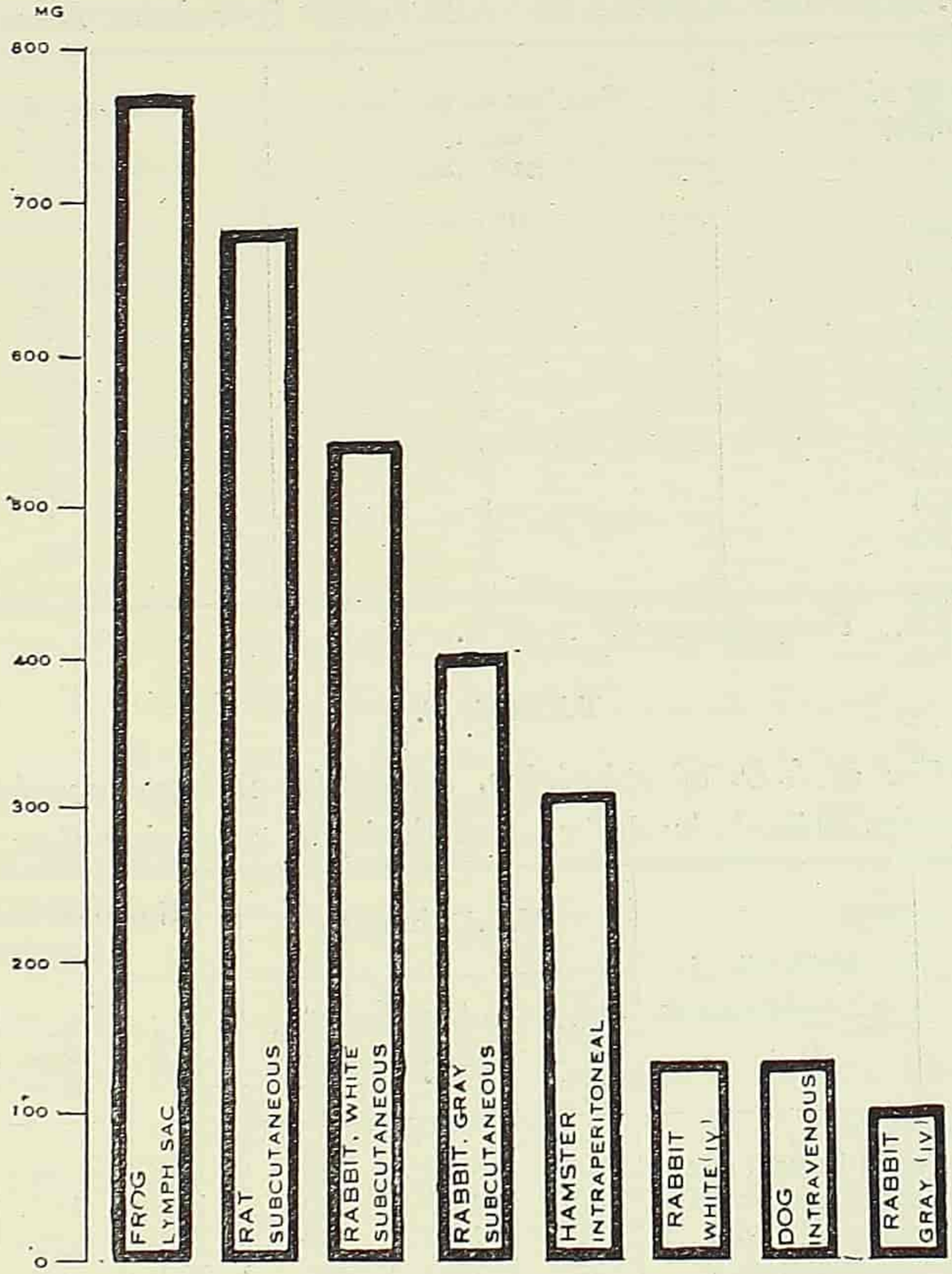

Fig. 3. Comparative magnitudes of M.L.D. of pseudoephedrine HCl in different animals by different methods of administration; (IV) intravenous. Dosage in milligrams per kilogram of body-weight.

III. The toxicity of pseudoephedrine.

So little work has been done upon pseudoephedrine that we made a fuller study of this isomer. One extra series of gray rabbits were used for intravenous injection. Table 8 shows how very toxic the effect can be, greater than for dogs or white rabbits. Figure 3 illustrates the comparative magnitude of the M.L.D. for various animals by

differing methods of administration. Pseudoephedrine is clearly less toxic than ephetonin and much less toxic than ephedrine, except in the hamster.

TABLE 8.

The M.L.D. of pseudoephedrine in white rabbits by subcutaneous injection

Dose per kg of body weight	Number of rabbits used	Number of rabbits died
mg		
320	1	0
350	2	0
500	7	1
525	2	0
540	3	2
550	3	3
570	2	2
650	1	1
700	1	1

October 1928. Temperature 22° to 25°C.

TABLE 9.

The comparative M.L.D. of ephedrine, ephetonin and pseudoephedrine in different animals by different methods of administration

Animals	Methods of administration	M.L.D. in mg			Ratio of M.L.D. in per cent		
		Ephedrine	Ephetonin	Pseudoephedrine	Ephedrine	Ephetonin	Pseudoephedrine
Frogs	into lymph sac	540	630	770	100	86	70
Hamster	intra-peritoneal	350	310	310	100	116	116
Rats	subcutaneous	320	350	680	100	91	47
Gray rabbits	subcutaneous	230	360	400	100	64	56
Gray rabbits	intravenous	80	90	100	100	89	80
White rabbits	intravenous	50	70	130	100	70	38
Dog	intravenous	70	100	130	100	70	54

IV. Symptomatology.

There are no new observations to report for the animals used by Amatsu and Kubota (1) and Chen (4).

In the hamster the three isomers show marked differences. Ephedrine is most convulsant in its effects, ephetonin less so and with pseudoephedrine one often saw only a slight tremor of the body. In other respects they are similar in effect.

Three to five minutes after administering ephedrine the animal becomes restless and is easily excited. Defecation, urination and efforts to vomit follow almost immediately and in ten to fifteen minutes the whole body trembles, especially the head. There is erection of the tail and hair and very soon there follows a series of violent convulsions. The animals often scream and leap high in the air. They are very sensitive to mechanical stimuli. The excitement and convulsions continue a long time. After thirty to sixty minutes the convulsions gradually decrease, the animal collapses on its abdomen and respiration becomes irregular and slow. Death occurs in 1 to 24 hours.

While pseudoephedrine produces similar symptoms the period of excitement is short, the animal soon lies on its abdomen and shows extreme difficulty in breathing. Death occurs in 20 to 60 minutes.

DISCUSSION

Our results in general show that in frogs, rats, rabbits and dogs ephedrine is definitely more toxic than ephetonin and pseudoephedrine. Our M.L.D. of ephedrine in the white rabbit intravenously confirms the result of Kreitmair. We do not agree with Chen's results (3) which show an identical toxicity for ephedrine and ephetonin.

In comparing ephedrine with pseudoephedrine our results are similar to those obtained by Fujii (6), though the ratio is nearer 1.4 than 1.6, as found by a little more careful measurement. From our knowledge of these drugs and the symptoms observed we conclude that in most animals death is due primarily to collapse of the heart due to depression of the muscle. In the hamster there is so much respiratory embarrassment that failure of respiration would appear to play a more important rôle as a cause of death. We suggest this as a possible explanation for the results from mice, in which one sees a similar inversion of the proportionate toxicity of ephedrine and ephetonin.

Seeing that ephetonin is not so strong or toxic a drug as ephedrine, we recommend that due consideration be given to adjustment of its therapeutic dose, and in comparing the minor toxic effects of these two isomers a proportionate dosage be taken as a basis of judgment regarding their untoward effects.

SUMMARY

1. The comparative toxicity of ephedrine, ephetonin and pseudoephedrine, as tested in frogs, rats subcutaneously, gray rabbits intravenously and subcutaneously, white rabbits and dogs intravenously, is in

the proportion of 100 to 78 to 56½ respectively. Ephedrine is more toxic than ephetonin, and the latter is more toxic than pseudoephedrine.

2. By intraperitoneal injection in the hamster the M.L.D. was 350, 310 and 310 mg respectively. This result as regards ephedrine and pseudoephedrine is similar to previous reports upon mice.

3. The M.L.D. of pseudoephedrine for frogs is 770 mg, for rats subcutaneously 680 mg, for gray rabbits subcutaneously 400 mg, intravenously 100 mg, for white rabbits intravenously 130 mg, and for dogs intravenously 130 mg.

4. Intravenously in the white rabbit the M.L.D. for ephedrine and ephetonin, are 50 and 70 mg respectively, which are identical with the ratio obtained in dogs, and even less than that in gray rabbits injected subcutaneously which require to produce death 230 and 360 mg, a ratio of 100 to 64. These figures contradict the statement that they are identical.

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麻黃素，假性麻黃素與愛佛託寧 Ephetonin 三者之比較。

其二. 毒性之比較

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用蛙，鼠，(皮下注射)，灰色兔(靜脈及皮下注射)白兔及狗 (靜脈注射)試驗證明毒性以麻黃素爲最強，Ephetonin 次之，假性麻黃素又次之；比例約爲一〇〇：七八：五六。五

若注入灰色鼴鼠(hamster) 之腹腔，則麻黃素之致死量爲三百五十公絲，假性麻黃素與 Ephetonin 則各爲三百一十公絲。

假性麻黃素之致死量，對于蛙爲七七〇公絲，對于鼠(皮下注射)則爲六八〇，對于灰色兔則爲四〇〇(皮下注射) 或一〇〇公絲 (靜脈注射)，對于白兔，則爲一三〇 (靜脈注射)，對于狗則每公斤體重，需七〇公絲。

靜脈注射之致死量，對于白兔則麻黃素爲五〇公絲，而 Ephetonin 爲七〇公絲。對于灰色兔皮下注射之致死量，則麻黃素爲二三〇而 Ephetonin 爲三六〇；此爲百與六十四之比，可見麻黃素與 Ephetonin 之不同。

Chinese Journal of Physiology. 1929, Vol. III, No. 1, pp. 95—108.

COMPARATIVE STUDIES OF EPHEDRINE, RACEMIC EPHEDRINE AND PSEUDOEPHEDRINE*

III. EFFECTS ON THE NASAL MUCOUS MEMBRANES

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Ephedrine is absorbed easily and quickly from the nasal mucous membrane. By either intravenous injection or local application, ephedrine produces a strong and sustained constriction of the nasal vessels. This effect has been demonstrated by King and Pak (3) to be much longer than that obtained with adrenalin or cocaine under similar circumstances. Berger and Ebster (1) found in asthmatic patients that the same dose of ephetonin has a slower and weaker effect than ephedrine, sometimes it required two to three times more ephetonin than ephedrine to produce the desired effect. However untoward symptoms from ephetonin were observed by them to be less frequent than from ephedrine. They used a 5 per cent spray in the treatment of acute infectious rhinitis and hay fever. Gay and Herman (2) in their preliminary report declared that the dose of ephetonin required to relieve asthmatic attacks, was greater than that of the natural ephedrine.

Pak and Read (5, 6) have demonstrated that ephetonin is less active than ephedrine upon the blood pressure, also showing that the latter is more active upon the sympathetic nervous system, and the former is more active upon muscle; moreover they found the M.L.D. for frogs, rats, rabbits and dogs was not the same, ephedrine being about 30 per cent stronger than ephetonin.

Seeing that there is this qualitative and quantitative difference in the action of these two isomers, it was highly desirable to demonstrate

*Preliminary communication read before the Chinese Physiological Society, Peking, January, 1928.

along the lines of our earlier work with ephedrine (3), their comparative effects upon the nasal mucous membranes.*

METHOD

Dogs anesthetized with luminal (0.18 gm per kg) were used throughout the experiments. The modified nasal plethysmographic method of Tschalussow, described in our previous paper (3) was employed to determine the volume change of the nasal cavity. A fall in the tracing denoted an increased nasal volume resulting from shrinkage of the nasal mucous membrane. The blood pressure was recorded from the left carotid artery. The drugs (1 cc of the solution and 2 cc saline) were injected into the femoral vein.

A preliminary trial was made of the effect of repeated dosage of the drug. It is well-known that successive doses do not produce the same effect on blood pressure on account of increasing depression of the heart muscle. It is not known whether the neurotropic effect upon the nose can be repeatedly demonstrated without similar decreases.

In the comparative experiments the relative maximal effects of only two drugs were determined by three injections. The third injection was considered merely a control of the first one. Although its effect was not quantitatively identical with the first injection, its action was similar to it, and did not resemble the second injection made with the isomer to be compared. An interval of three hours was allowed between each of the injections. The average dosage was about 0.2 mg per kg of body weight. In estimating the relative dimensions of the nasal volume following injection of the drug, measurements were made on the perpendicular line drawn between the initial level and the lowest level of the curve produced. Because the nasal volume has been proved to be extremely sensitive to vasoconstrictor drugs, as observed by Moltschanow (4) who employed the changes in nasal volume for the bioassay of adrenalin and found that dilutions as high as 3×10^{-8} when injected directly through the carotid could repeatedly produce a considerable increase in the nasal volume, we tried to produce the same result with ephedrine by injection through a cannula attached to the carotid artery. A noticeable increase of the nasal volume was successfully obtained with ephedrine 1×10^{-5} but the responses of the nasal

*Since presenting this paper Chen has published work showing the effect of ephetonin on nasal volume, and he quotes Slack as being "unable to detect any significant difference between their constricting power". Chen, K. K., J. Pharm. Exper. Therap., 1928, **33**, 237-258.

mucosa on repeated injections of the same dose were not identical. These variations might be due to delayed absorption of the drug from the circulation, or to varying reflex changes of nasal volume as a result of mechanical irritation of the artery when the drug was being injected. Consequently we adopted intravenous injections of the drugs. Three preliminary experiments were tried for establishment of the method by experimentation with the same and different doses of ephedrine in order to be sure whether the action upon nasal volume was proportionate to the dosage used.

RESULTS

I. The effects produced by repeated doses of ephedrine.

In one experiment three repeated injections of 0.2 mg per kg at two hour intervals yielded approximately equal nasal responses. Another experiment upon a dog weighing 13.6 kg, was made with a larger dose and the following results were obtained.

	B.P.	N.V.
Ephedrine 2 mg	28 mm Hg	25 mm
,, 4 ,,	42 ,,	51 ,,
,, 2 ,,	18 ,,	24 ,,

Both experiments clearly demonstrated increase of nasal volume to correspond almost exactly with the increase of the dosage of ephedrine, this is true although there is seen the usual marked differences in the blood pressor effects (see table 1).

II. Comparison of ephedrine and ephetonin. Seven dogs, see fig. 1.

a. Nasal volume.

Dosage: 0.17 to 0.23 mg per kg of body weight.

Nasal volume increased simultaneously with shrinkage of the mucosa of the turbinated bodies, and the nasal accessory sinuses. Nasal volume began to increase immediately after administration of each drug. In the case of ephetonin it reached the maximal effect in 35 to 80 sec., whereas in the case of ephedrine the maximal effect took place after 40 to 120 sec. The difference is due to the fact that while ephedrine in the same time may cause the same degree of shrinkage as ephetonin it is stronger and thus longer in reaching its maximal effect. The effects on blood pressure and shrinkage of mucosa did not go in parallel. The maximal rise of blood pressure was obtained 20 to 30 sec. after intravenous injection of ephedrine, and after

TABLE 1.

Proportional effect on the nasal volume of increasing doses of ephedrine

Time	Drugs (intraven. injection)	Blood pressure	Maximum effect of B.P.	Nasal volume	Maximum effect of N.V.
		mm Hg	*mm Hg*		*mm**
	Control	78			
11:12	Ephedrine HCl, 1 mg (=.089 mg per kg)				
11:12:30		92	+14	increasing	
11:13:25		90		,,	+19
11:15		90		returning	
1:14		102			
1:15	Ephedrine HCl, 2 mg (=.178 mg per kg)				
1:15:30		122	+20	increasing	
1:17		110		,,	+31
1:18		100		returning	
3:18		104			
3:19	Ephedrine HCl, 3 mg (=.268 mg per kg)				
3:19:35		128	+24	increasing	
3:21:35		110		,,	+61

Sept. 12, 1927. Dog ♂, 11.4 kg, luminal (0.18 gm per kg of body weight) subcutaneously.

*The number of mm is taken from the measurement of the distance between the original level and the tangent of the curve, which represented the change of nasal volume (N.V.).

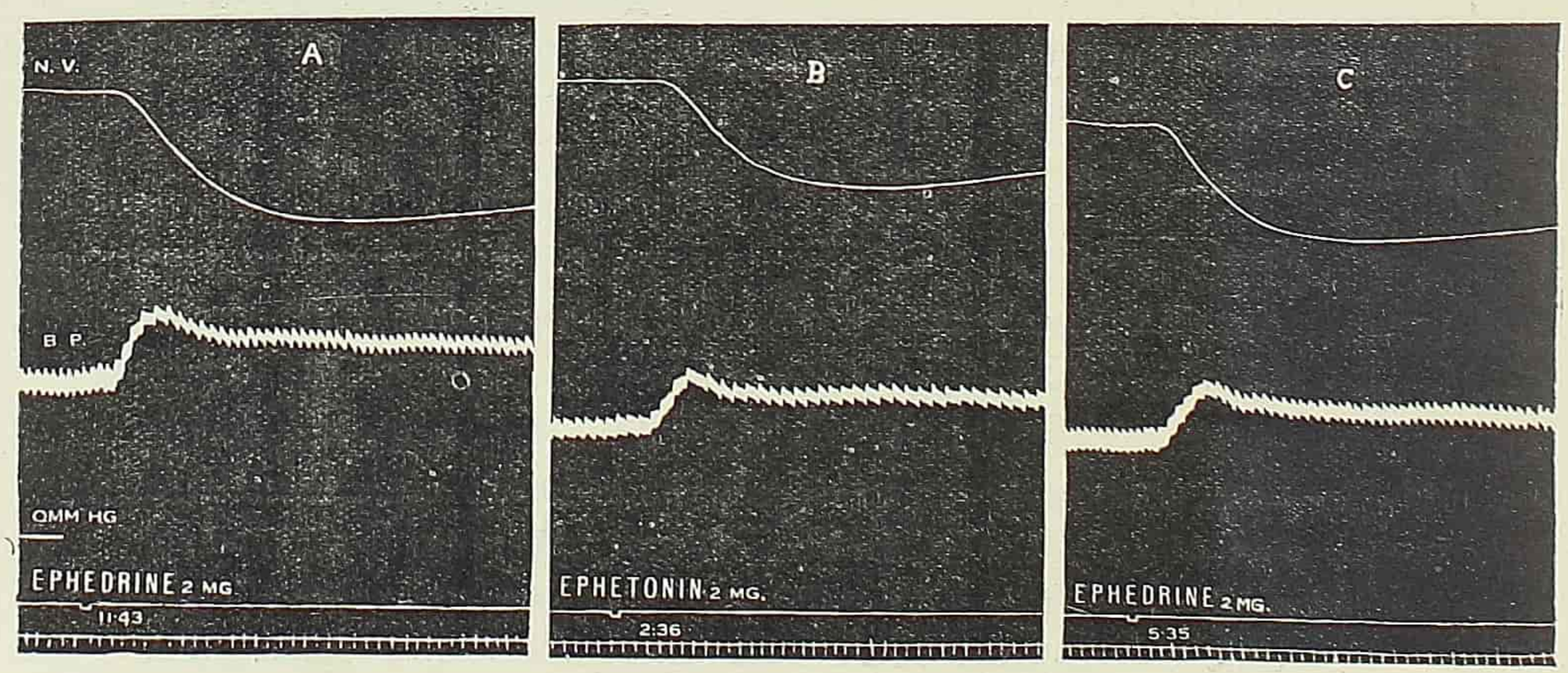


Fig. 1. Comparative effects of ephedrine and ephetonin on the nasal mucous membrane. December 15, 1927. Dog ♂, 11.8 kg, luminal (0.18 gm per kg) subcutaneously). N.V.—Nasal volume. The fall in tracing indicates an increased nasal volume resulting from shrinkage of the mucosa. B.P.—Blood pressure.

(A) 11:43 Ephedrine HCl 2 mg intravenously: 34 mm Hg rise in B.P.; 35 mm fall in N.V. tracing. (B) 2:36 Ephetonin HCl 2 mg intravenously: 26 mm Hg rise in B.P.; 29 mm fall in N.V. tracing. (C) 5:35 Ephedrine HCl 2 mg intravenously: 29 mm Hg rise in B.P.; 32 mm fall in N.V. tracing.

10 to 30 sec. for ephetonin. At the time when the blood pressure reached its maximum the nasal volume continued to increase, and reached its maximum during the returning phase of the blood pressure rise. See table 2.

When ephedrine was injected previous to ephetonin the ratio of *maximal shrinkage* was:

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.76 \text{ (Average from 4 experiments).}$$

When ephetonin was injected first and ephedrine afterwards, the ratio was:

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.75 \text{ (Average from 2 experiments).}$$

Average ratio:

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.76.$$

Figure 1 demonstrates a typical experiment.

The duration, after small doses of 0.2 mg per kg, of the shrinkage of the nasal mucosa is not the same, it being more prolonged in the case of ephedrine than with ephetonin in the ratio of 1 to 0.74. Three minutes after intravenous injection of ephedrine the increased nasal volume was gradually lowered to 93 per cent of the maximal effect and to 69 per cent when ephetonin was used.

b. Blood pressure.

In the same experiments with a dosage of 0.17 to 0.23 mg per kg, when ephedrine was injected before ephetonin, we obtained the following ratios of blood pressure effect.

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.53 \text{ (Average from three experiments).}$$

When ephetonin was given before ephedrine:

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.83 \text{ (Average from three experiments).}$$

Average ratio:

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.68.$$

Three minutes after injection the blood pressure came down to 68 per cent of the maximal rise in the case of ephedrine and to 34 per cent for

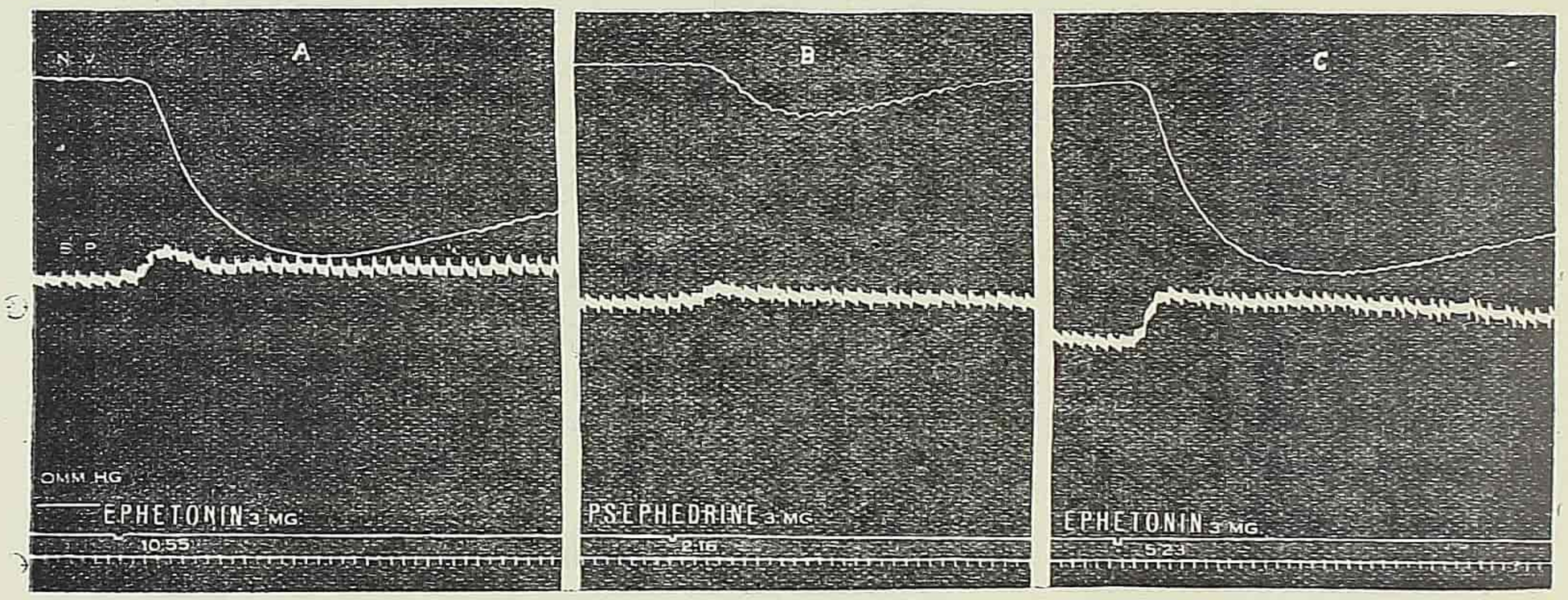


Fig. 2. Comparative effects of ephetonin and pseudoephedrine on the nasal mucous membrane. November 17, 1927. Dog ♂, 13.2 kg, luminal (0.18 gm per kg) subcutaneously.

(A) 10:55 Ephetonin HCl 3 mg intravenously: 16 mm Hg rise in B.P.; 50 mm fall in N.V., tracing. (B) 2:16 Pseudoephedrine HCl 3 mg intravenously: 8 mm Hg rise in B.P.; 15 mm fall in N.V. tracing. (C) 5:23 Ephetonin HCl 3 mg intravenously: 26 mm Hg rise in B.P.; 52 mm fall in N.V. tracing.

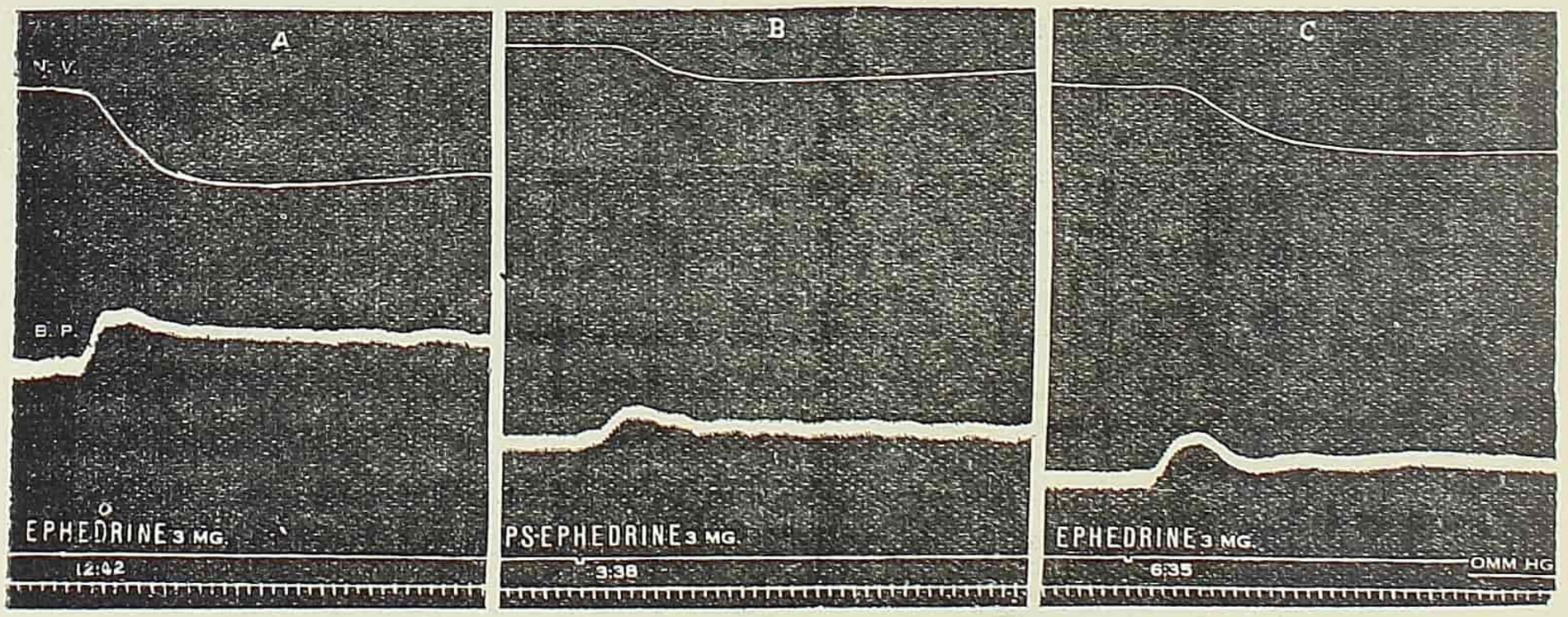


Fig. 3. Comparative effects of ephedrine and pseudoephedrine on the nasal mucous membrane. November 24, 1927. Dog ♂, 12.8 kg, luminal (0.18 gm per kg) subcutaneously.

(A) 12:42 Ephedrine HCl 3 mg intravenously: 26 mm Hg rise in B.P.; 27 mm fall in N.V. tracing. (B) 3:38 Pseudoephedrine HCl 3 mg intravenously: 16 mm Hg rise in B.P.; 10 mm fall in N.V. tracing. (C) 6:35 Ephedrine HCl 3 mg intravenously: 22 mm Hg rise in B.P.; 18 mm fall in N.V. tracing.

ephetonin. The blood pressure effect is much more prolonged in the case of ephedrine than ephetonin. The ratio of the *duration* of the blood pressor action is:

$$\frac{\text{Ephetonin}}{\text{Ephedrine}} = 0.5$$

III. Comparison of ephetonin and pseudoephedrine. Four expt. see fig. 2.

a. Nasal volume.

The maximal shrinkage produced by pseudoephedrine was reached 35 to 70 sec., after injection.

When pseudoephedrine was injected before ephetonin, the ratio was:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.68 \text{ (Average from two experiments).}$$

When ephetonin was injected before pseudoephedrine:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.57 \text{ (Average from two experiments).}$$

Average ratio:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.63$$

Three minutes after injection the increased nasal volume returned to 75 per cent of the maximal effect in the case of ephetonin and 61 per cent for pseudoephedrine. The duration of the effect of shrinkage of the mucosa was greater with ephetonin than with pseudoephedrine in the following ratio:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.81$$

b. Blood pressure.

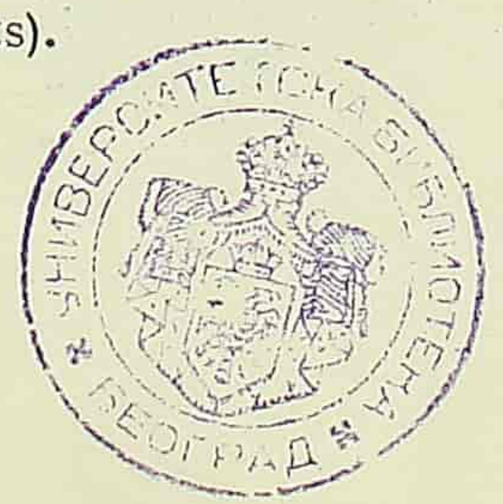
Pseudoephedrine is similar to ephedrine and ephetonin in not producing parallel effects upon blood pressure and nasal volume, see figs. 2 and 3 and table 3.

When ephetonin was given before pseudoephedrine:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.44 \text{ (Average from two experiments).}$$

When pseudoephedrine was given before ephetonin:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.68 \text{ (Average from two experiments).}$$



Average ratio:

$$\frac{\text{Ps-ephedrine}}{\text{Ephetonin}} = 0.56.$$

Three minutes after injection of ephetonin the blood pressure decreased to 52 per cent of the maximal rise, and 37 per cent in the case of pseudoephedrine. The effect of blood pressure is more prolonged in the case of ephetonin than that of pseudoephedrine in the ratio of 1 to 0.7.

IV. The comparison of ephedrine and pseudoephedrine. See fig. 3.

a. Nasal volume. Two experiments, see table 4.

When ephedrine was given before pseudoephedrine:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.37$$

When pseudoephedrine was given before ephedrine:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.38.$$

Average ratio:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.38.$$

The ratio of the duration of effect was:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.7.$$

Three minutes after the injection of ephedrine the increased nasal volume decreased to 85 per cent of the maximal effect and 60 per cent for pseudoephedrine.

b. Blood pressure

If ephedrine was given before pseudoephedrine.

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.62$$

If pseudoephedrine was given before ephedrine:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.50$$

Average ratio:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.56$$

The ratio of duration of effect was:

$$\frac{\text{Ps-ephedrine}}{\text{Ephedrine}} = 0.40.$$

Three minutes after injection of ephedrine the blood pressure diminished to 62 per cent of the maximal rise of blood pressure and to 25 per cent for pseudoephedrine.

V. The comparison of ephedrine, ephetonin and pseudoephedrine.

Combining the results obtained in II, III and IV we deduce the following ratios:—

	Ephedrine	Ephetonin	Pseudoephedrine
a. Maximal effect on nasal volume:	1	0.76	0.38
	1.32	1	0.50
	2.67	2.01	1
b. The duration of shrinkage of the mucosa:	1	0.80	0.68
c. Blood pressure effect of small doses (0.2 mg per kg):	1	0.68	0.56
d. The duration of the blood pressor action:	1	0.66	0.48

DISCUSSION

Seeing that ephetonin does not have as great an effect in contracting the nasal mucous membranes, when applied locally to obtain a maximum effect more is needed than when ephedrine is used. This suggests a further study of the local action of varying concentrations of these drugs.

The fact that there is definite contraction of the membranes both with ephetonin and pseudoephedrine, makes it possible to substitute them for ephedrine should the latter drug have undue untoward effects or in any other way be contraindicated.

CONCLUSIONS

1. Like ephedrine both pseudoephedrine and ephetonin cause a distinct and sustained shrinkage of the nasal mucous membrane.

2. The relative strengths of the vasoconstrictor effect of the three isomers are:

Ephedrine: Ephetonin: Pseudoephedrine = 1: 0.76: 0.38.

3. Their durations of shrinkage has the ratio:

Ephedrine: Ephetonin: Pseudoephedrine = 1: 0.8: 0.7.

4. The nasal plethysmographic method is suitable for the bio-assay of the vasoconstrictor action of ephedrine.

TABLE 2.

Comparison of the maximal effects of small doses of ephedrine and ephetonin upon the nasal volume and blood pressure of dogs

(a) Ephedrine injected first

Dose per kg mg	Order of injections	Increases in nasal volume				Increases in blood pressure			
		Maximal effect	After 3 min.	1. Ephedrine 2. Ephetonin	2. Ephetonin 1. Ephedrine	Maximal effect	After 3 min.	1. Ephedrine 2. Ephetonin	2. Ephetonin 1. Ephedrine
0.2	Dog ♂, 15.9 kg	*mm**	*mm*			*mm Hg*	*mm Hg*		
	1. Ephedrine	55	52	1.22	0.82	30	22	3.00	0.33
	2. Ephetonin	45	37			10	6		
	3. Ephedrine	50	46			18	12		
0.17	Dog ♂, 11.8 kg								
	1. Ephedrine	35	32	1.21	0.83	34	22	1.30	0,76
	2. Ephetonin	29	24			26	16		
	3. Ephedrine	32	27			29	17		
0.2	Dog ♂, 10 kg								
	1. Ephedrine	54	50	1.35	0.74	43	29	1.95	0.51
	2. Ephetonin	40	34			22	16		
	3. Ephedrine	36	35			22	18		
0.2	Dog ♂, 9.2 kg								
	1. Ephedrine	17	11	1.55	0.65	—	—	—	—
	2. Ephetonin	11	8			22	8		
	3. Ephedrine	14	9			24	12		

*The number of mm is taken from the measurement of the distance between the original level and the tangent of the curve, which represented the change of nasal volume.

TABLE 2.—*(Concluded)*

(b) Ephetonin injected first

Dose per kg mg	Order of injections	Increases in nasal volume				Increases in blood pressure			
		Maximal effect	After 3 min.	2. Ephedrine / 1. Ephetonin	1. Ephetonin / 2. Ephedrine	Maximal effect	After 3 min.	2. Ephedrine / 1. Ephetonin	1. Ephetonin / 2. Ephedrine
0.2	Dog ♂, 10.9 kg	*mm**	*mm*			*mm Hg*	*mm Hg*		
	1. Ephetonin	—	—	—	—	24	8	1.67	0.60
	2. Ephedrine	25	21			**40**	22		
	3. Ephetonin	17	13			28	8		
0.17	Dog ♂, 11.6 kg								
	1. Ephetonin	37	28	1.24	0.80	16	4	1.13	0.89
	2. Ephedrine	46	40			18	8		
	3. Ephetonin	36	28			16	6		
0.2	Dog ♀, 8.7 kg								
	1. Ephetonin	26	16	1.42	0.70	14	6	1.00	1.00
	2. Ephedrine	37	24			14	14		
	3. Ephetonin	18	11			12	10		
Av.				(a) 1.33	(a) 0.76			(a) 2.08	(a) 0.53
Av.				(b) 1.33	(b) 0.75			(b) 1.27	(b) 0.83

TABLE 3.

Comparison of the maximal effects of small doses of ephetonin and pseudoephedrine upon the nasal volume and blood pressure of dogs

Dose per kg mg	Order of injections	Increases in nasal volume				Increases in blood pressure			
		Maximal effect	After 3 min.	Ephetonin / Ps-ephed.	Ps-ephed. / Ephetonin	Maximal effect	After 3 min.	Ephetonin / Ps-ephed.	Ps-ephed. / Ephetonin
0.2	Dog ♂, 13 kg	*mm**	*mm*			*mm Hg*	*mm Hg*		
	1. Ps-ephedrine	33	26	(a) 1.18	0.85	5	2	2.4	0.42
	2. Ephetonin	39	38			12	8		
	3. Ps-ephedrine	4	4			7	2		
0.2	Dog ♂, 8.9 kg								
	1. Ps-ephedrine	9	6	2.00	0.50	15	4	1.07	0.94
	2. Ephetonin	18	11			16	0		
	3. Ps-ephedrine	3	0			14	5		
0.2	Dog ♀, 9.4 kg								
	1. Ephetonin	12	8	(b) 1.20	0.83	16	4	2.67	0.38
	2. Ps-ephedrine	10	8			6	4		
	3. Ephetonin	17	9			10	2		
0.23	Dog ♂, 13.2 kg								
	1. Ephetonin	50	38	3.33	0.30	16	8	2.00	0.50
	2. Ps-ephedrine	15	3			8	2	1.	
	3. Ephetonin	52	41			26	18	3.25	
Av.				(a) 1.59	0.68			1.74	0.68
Av.				(b) 2.27	0.57			2.34	0.44

*The number of mm is taken from the measurement of the distance between the original level and tangent of the curve which represented the change of nasal volume.

TABLE 4.

Comparison of the maximal effects of small doses of ephedrine and pseudoephedrine upon the nasal volume and blood pressure of dogs

Dose per kg mg	Order of injections	Increases in nasal volume				Increases in blood pressure			
		Maximal effect	After 3 min.	1. Ephedrine 2. Ps-epned.	2. Ps-ephed. 1. Ephedrine	Maximal effect	After 3 min.	1. Ephedrine 2. Ps-ephed.	2. Ps-ephed. 1. Ephedrine
0.23	Dog ♂, 12.8 kg	*mm**	*mm*			*mm Hg*	*mm Hg*		
	1. Ephedrine	27	23	2.70	0.37	26	16	1.63	0.62
	2. Ps-ephedrine	10	7			16	4		
	3. Ephedrine	18	17			22	8		
0.2	Dog ♀, 5.5 kg								
	1. Ps-ephedrine	5	3	2.60	0.38	8	2	2.00	0.50
	2. Ephedrine	13	10			16	10		
	3. Ps-ephedrine	3	3			4	2		

*The number of mm is taken from the measurement of the distance between the original level and the tangent of the curve, which represented the change of nasal volume.

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麻黃素，假性麻黃素與愛佛託寧 Ephetonin 三者之比較。

其三。對于鼻粘膜之作用

金　鑄　　朴杜秉

北京協和醫學校耳鼻喉學系及藥物學系，北平.

假性麻黃素，麻黃素與 Ephetonin 均能使鼻粘膜收縮頗久。

此三者之血管收縮作用，其強弱，不同，若以麻黃素爲單位，則 Ephetonin 爲百分之七十六，而假性麻黃素爲百分之三十八。

此三者之血管收縮作用，其久暫亦不同，若以麻黃素爲單位，則 Ephetonin 爲十分之八，而假性麻黃素爲十分之七。

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