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CONTRIBUTIONS TO THE PHYSIOLOGY OF LYMPH SECRETION.

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I. On the mode of action of Lymphagogues.

In a recent paper[1] Heidenhain has called attention to the existence of two classes of bodies, which, when injected into the circulation, have the power of largely increasing the flow of lymph. The first class, which includes peptone, leech-extract, extract of crayfish and mussels, and under some conditions, egg-albumen, and decoction of intestinal wall and liver, seems to act particularly in increasing the exudation from the blood, rendering this more concentrated, at the same time that the flow of lymph is augmented. The second class, which includes crystalline bodies, such as sugar and neutral salts, acts on the tissues, robbing these of water, so that both lymph and blood are rendered more watery.

The present investigation deals entirely with the mode of action of the bodies belonging to the first class. On injection of peptone into the blood, not only is the flow of lymph increased, but also the amount of its solid constituents[2]. At the same time, the solid constituents of the blood-plasma are diminished, so that we must conclude that a fluid more concentrated than the plasma transudes through the vessel-walls. It is chiefly this fact which supports the idea that the increased formation of lymph after peptone-injection is due to an active process of secretion by the endothelial cells of the capillary walls.

These bodies, however, have another property in common. All of

[1] "Versuche und Fragen zur Lehre von der Lymphbildung." *Pflüger's Archiv*, Bd. XLIX. 1891, p. 1.

[2] *loc. cit.* p. 44.

them to a greater or less degree alter the blood and diminish or destroy its coagulability. This is especially the case with peptone, leech-extract and extract of crayfish and mussels. In the case of some other substances, such as casein and foreign serum, which, as I have found, have an inconstant effect on the lymph, but which appear to be also members of the first class (from their simultaneous effect on lymph and plasma), diminution of coagulability of the blood occurs at the same time as increased flow of lymph.

The question naturally arises whether in all these cases the real stimulus to the endothelial cells may not lie in the altered blood itself and not in the substance injected. In this event, the primary action of peptone injection would be an alteration of the blood, and the increased transudation or secretion of lymph would be immediately caused by the alteration of the blood and would be only a secondary effect of the peptone, and in the same way with the other members of this class of lymphagogues. The experimental solution of this question in one direction or another would seem to be an easy task. If the alteration of the blood is the cause of the increased lymph-flow, the injection into a dog of a large amount of blood altered by peptone, but containing little or none of this body, should produce a flow of lymph similar to that brought about by the direct introduction of peptone. Peptone is chosen as an example of the first class, since it is the only one whose chemical characters are well defined, and for which we have definite chemical tests,—so that it should be easy to ascertain whether a given specimen of blood, altered by the injection of peptone, contains an appreciable amount of this body or not. Moreover, according to all who have worked at this subject, peptone disappears extremely rapidly from the blood after its injection, so that we may get blood altered by peptone, but containing none of this body.

We must, in the first place, know the effect that the mere increase of the blood in the vascular system of an animal will have on the lymph, and, secondly, the result of injecting blood that has been made uncoagulable by other means than the administration of peptone or leech-extract—as, for example, whipped blood or serum or oxalated blood. Since these control experiments are in themselves interesting, I quote some of them here.

1. *Effect of transfusing normal blood.*

Two experiments were performed. The carotid artery of one dog, *A*, was connected by means of a glass cannula with the facial vein of a

second dog, *B*. A cannula was placed in the thoracic duct of *B*, and the blood-pressure of *B* was registered by means of a Hürthle's manometer, connected with a femoral artery.

Experiment 1. *April 26th*, 1892.

Dog *A* weighs 8,000 grms. Dog *B* 7,000 grms.

Lymph from *B* in every 10 minutes from 10.32 to 11.35 a.m.:—

4 c.c. 4 c.c. 3·25 c.c. 2·5 c.c.

From 11.42 to 11.52 blood allowed to flow from *A* into *B*, the artery being held between the finger and thumb, so that the flow of blood could be accurately controlled. Care was taken not to let the transfusion take place too rapidly, only small amounts of blood being allowed to flow in at a time, by releasing the compression on the artery for a second and then closing it again. The blood-pressure in *B* rose slightly. By comparing the weight of *A* before and after experiment, it was calculated that 350 grms. had been transfused. Lymph in every 10 minutes after transfusion from 11.52 to 12.32:—

5 c.c. 5 c.c. 4·5 c.c. 4·5 c.c.

Experiment 2. *June 1st*, 1892.

A weighs before transfusion 5510 grms.

A weighs after „ 5260 „

The amount of blood transfused therefore = 250 grms.

B weighs 12·450 grs.

Lymph from *B* in each 10 min. from 10.40 to 11 a.m.:—

6 c.c. 5 c.c.

From 11 to 11.9 blood allowed to flow from *A* into *B*. Blood-pressure in *B* rose considerably.

Lymph from *B* in every 10 min. from 11.10 to 11.40:—

8·5 c.c. 9 c.c. 10 c.c.

2. *Effect of transfusing defibrinated blood.*

In Experiment 2, at 11.45 a.m. the dog *B* was bled to 220 c.c. The blood was whipped and strained. The effect of the bleeding was to cause a slight decrease in the lymph.

Thus in each 10 min. from 11.47 to 12.7, the lymph amounts were:—

7·5 c.c. 5 c.c.

From 12.8 to 12.18 p.m., the defibrinated blood was allowed to flow in again by the facial vein.

The lymph amounts in each 10 min. from 12.17 to 12.37 were:—

7·5 c.c. 7·5 c.c.

Two other experiments with the introduction of defibrinated dog's blood had similar results.

If defibrinated blood or serum of another species be introduced, the results are not so constant. Five experiments were made with the introduction of defibrinated blood (3 cases) and of serum (2 cases) from the ox. In three of these experiments a very large increase in the lymph-flow was produced; in two, the effect was minimal and could be ascribed to the mere increase in bulk of the circulating fluid. It is evident that the ox-serum in some cases may contain a body which acts as a lymphagogue, but which is not invariably present.

3. *Introduction of oxalated dog's blood.*

I.e., blood that has been received directly into a solution of sodium oxalate, so that the whole mixture shall contain 2 ‰ of the oxalate (Arthus).

Two experiments of this sort were performed.

Experiment 4. *June* 20*th*, 1892.

Dog 8·720 grms.

Lymph—from 10.30 to 10.50 = 3 c.c.
from 10.50 „ 10.55 = 1 c.c.

From 10.55 to 11.5, 150 c.c. oxalate blood from another dog was slowly injected by cannula in facial vein.

Lymph—from 11.5 to 11.15 = 5 c.c.
from 11.15 „ 11.25 = 3·5 c.c.
from 11.25 „ 11.35 = 2·5 c.c.
from 11.45 „ 11.55 = 2 c.c.

From 11.45 to 11.55, 160 c.c. more oxalate blood injected.

from 11.55 to 12.5 = 7 c.c.
from 12.5 „ 12.15 = 4·5 c.c.
from 12.15 „ 12.25 = 4 c.c.
from 12.25 „ 12.35 = 3·5 c.c.

In another experiment (Exp. 5, June 30, 1892) the lymph in the 10 minutes before the injection of 150 c.c. oxalate blood was 3·5 c.c. In the 10 minutes' periods afterwards it was:—

5 c.c. 5 c.c. 4·5 c.c.

In no case did introduction of dog's defibrinated blood, of normal

blood or of oxalate blood alter in any way the coagulability of the blood of the receiving animal.

We see, then, that the effect of increasing the volume of the circulating fluid is to cause a slight and transitory increase in the flow of lymph. In no case, however, was the lymph-amount after injection more than double the previous amount, and of this, part must be ascribed to the lymph mechanically squeezed out of the extravascular lymph-spaces by the vascular dilatation.

We may now proceed to the experiments in which the peptonised blood was injected into the circulation.

For convenience of reference, I have arranged them in three tables. With regard to the first two classes, in which the peptonised blood was injected into a vein (generally the facial) it is necessary to mention certain sources of error that may give rise to faulty conclusions from experiments, and the importance of which I only recognised after many experiments.

In the first place, the injection must be carried out slowly and under low pressure. If the facial vein of one dog be connected with the carotid artery of the other, and blood allowed to flow in unchecked, the right heart becomes engorged with more blood than it is able to expel, and the blood becomes dammed up, causing large rise of pressure in the inferior vena cava, with tremendous congestion of the liver, accompanied by bloody extravasations round the gall-bladder.

Heidenhain has shown that obstruction of the inferior vena cava gives rise to a large increase of lymph, and I have observed that such obstruction as that caused by over-filling of the right heart gives rise to a large increase of lymph-flow, the lymph becoming very bloody. In one case in which the transfusion was allowed to take place too quickly, so that 300 c.c. blood were transfused in less than two minutes, the lymph looked almost like pure blood. The respiratory distress of the animal at the same time betokened the failure of the right heart to deal with the mass of blood that was thrown into it.

Another fallacy may arise from the fact that small clots may form in the facial vein at the junction with the cannula and be carried with the stream of injected blood into the inferior vena cava—there growing by accretion, and giving rise to blocking of the lumen of this vessel, and a purely mechanical increase of lymph. In one case, a clot had been carried into (or formed in) a hepatic vein, giving rise to an increased flow of lymph with a large admixture of blood. In all experiments, a careful examination was made, p.m., of all the large

vessels. These cases in which an increased flow of lymph was evidently due to a mechanical block (either enormous venous congestion or clot in the inferior vena cava or its branches) are not included in the tables.

Table I. includes three experiments in which transfusion was effected directly from one animal to another.

The carotid artery of one dog, *A*, was connected by a glass cannula, filled with normal salt solution, with the facial vein of another dog, *B*. The blood-pressure in *B* was registered by means of a manometer connected with one femoral artery, and the other femoral artery had a cannula for taking specimens of blood. A cannula was placed in the thoracic duct of *B*.

Peptone (0·5 grams. pro kilo.) was then injected into a femoral vein of *A*, and at a varying period after the injection blood was allowed to flow from *A* into *B* by releasing the clamp on the carotid *A*. I quote Experiment 2, because at the p.m. no mechanical blockage or excessive congestion of the liver could be found. The fact, however, that the lymph was very bloody immediately after the transfusion gives grounds for suspecting that here also a certain amount of damming up of the blood in the vena cava took place owing to the over-filling of the right heart. Of the other two experiments, in No. 1 there was no increase and in No. 3 the increase was not very pronounced, being from 1 to 2·5. No. 1 is worthy of attention since, owing to the cannula being too narrow at first, very little blood passed from *A* into *B* until it was changed, so that the chief transfusion occurred twenty minutes after the injection of peptone into *A*. There was no doubt here that the blood was still altered by the peptone since the blood both in *A* and *B* was found to be uncoagulable after the transfusion and yet this alteration in the blood was not accompanied by any increased flow of lymph.

In six experiments (Table II.) the dog *A* was bled at varying periods, two to eleven minutes after the peptone injection, and the blood obtained was allowed to flow from a graduated burette into the facial vein of *B*. Here, as in the first series, the same precautions as to slowness of transfusion, &c., must be taken. It will be seen that in all these cases the lymph was increased after the transfusion, although in four of them, this increase was only threefold. In No. 6, the increase was tenfold and in No. 9 fivefold, and so approximated that brought forth by the injection of peptone itself. It is worthy of note, however, that in both these cases the peptone had failed in effecting an appreciable alteration in the blood from *A* since in both these cases the blood from *A* clotted within twenty minutes after leaving the vessels.

Thus, out of these 14 experiments, there are only three cases (Exp. 2, 6 and 9) in which the increase in the flow of lymph was more than threefold, and of these, as above mentioned, No. 2 is dubious on account of the large amount of blood that appeared in the lymph after transfusion. In the two other cases, Nos. 6 and 9, the increased lymph-flow was brought about by the injection of blood that was apparently unaltered by the peptone injection, since it clotted shortly after leaving the vessels.

Heidenhain has shown that small doses of peptone may produce a large increase in the flow of lymph, if injected into the thoracic aorta, so that it is carried by the blood directly into the capillaries of the portal area. It was to be expected that if peptone blood,—that is to say, the altered blood and not peptone itself—were the real stimulus for the secretory activity of the endothelial cells, the injection of such blood into the aorta should have a greater effect on the lymph-flow than injection into a vein. At the same time, we avoid by this mode of experimenting, the dangers of creating artificial mechanical hindrances to the flow of blood, that are present when we transfuse into a vein.

Table III. gives the results on the lymph-flow of injecting peptone blood into the arterial system or aorta. In these five experiments, which may be regarded as perfectly free from mechanical complications, the greatest increase in the flow of lymph was threefold, in one experiment. In two other experiments the lymph-flow was doubled, and in two not materially altered. In Exp. 15, in which there was no increase in the flow of lymph, not only was the blood from *A* altered by the peptone injection, but it had also the power of altering the blood in *B* and rendering this too uncoagulable.

We see then, that in the above series of experiments the transfusion of the peptonised blood of dog *A* into dog *B* gave rise in two cases (6 and 9) to a very considerable increase in the lymph-flow. In most of the other cases, a moderate increase was observed, which, however, was greater than the increase brought about by injection of the same bulk of normal blood, or of defibrinated or oxalated blood.

The question arises then, whether this increase is dependent on the changed condition of the blood from the dog *A*, or whether perhaps enough peptone was still present in the transfused blood to give rise to the increase observed.

The first possibility, however, seems to be negatived by the fact that in the two cases 6 and 9, in which a great increase in the flow of lymph was produced, the transfused blood happened to be unaltered by

the peptone injection, and clotted in the cannula before the injection was quite finished. These two cases must be evidently put in the category of dogs which are immune to peptone, at any rate in ordinary doses. Fano[1] found that in 6 % of his dogs, peptone had no power to destroy the coagulability of the blood.

I may mention here too, that I have observed in some cases, after peptone injections, a large increase in the flow of lymph, although the blood, drawn immediately after the injection, clotted as soon or sooner than normal blood.

One experiment, in which gradually increasing amounts of peptone were injected, shows well this independence between the increased lymph-flow and the alteration in the blood. A dog weighing 9 kilos. was injected at intervals of about half-an-hour with 3 centigrams, 14 centigrams, 28 centigrams, 1 gram, and 5 grams peptone. The first four injections produced very little effect, either on lymph or blood. After the last injection of 5 grams, the lymph-flow was increased six-fold; the blood, however, that was drawn off directly after the injection clotted within four minutes, so that the blood did not lose its coagulability, although the lymph was markedly increased.

These facts certainly argue against the possibility that the loss of coagulability of the blood, in consequence of peptone injection, has anything to do with the increased lymph-flow which is at the same time called forth, and incline one to think that the lymphagogue action of the blood from the dog *A* may be due to a certain amount of peptone still present in this blood.

Certainly, all authorities are agreed that peptone disappears with extreme rapidity from the blood after injection, so that the amount present in the transfused blood can have only been extremely small.

I have, however, observed that a certain number of dogs are extremely sensitive to the action of peptone, so that very minute doses in them produce an effect on the lymph stream comparable with that produced in them normally by an injection of 0·3 to 0·5 grams pro kilo.

The following experiment affords a striking example of this abnormal susceptibility.

June 23. In a dog weighing 13 kilos, the amount of lymph in 10 minutes from 10.45 to 10.55 a.m. was 1·5 c.c.

A solution of 2 decigrms. peptone in 20 c.c. normal saline solution was

[1] *Du Bois' Archiv*, 1881.

then allowed to flow as slowly as possible into the facial vein. The injection lasted from 11.1 to 11.7. At 11.6 there was a sudden fall of blood-pressure, just as one observes in ordinary dogs after injection of the normal amount —0·3 grms. per kilo. At the same time, the lymph was enormously increased, so that from 11.7 to 11.11, 10 c.c. were obtained, or sixteen times the amount obtained in the same period before the injection. The specimen of blood that was drawn off after the injection was still fluid at 4 p.m. on the same day. This result is clearly anomalous, but it serves to show that just as there are dogs which are very little affected by large doses of peptone, so there are others extremely sensitive to minute doses of this substance. It is possible that the dogs in Experiments 6, and 9, in which peptone-blood produced a large increase in the lymph, were also more than normally sensitive to the influence of small doses of peptone.

In other experiments, in which small doses of peptone were injected a slight increase was brought about. In one experiment, on June 25th, one decigram was injected into a dog weighing 6 kilos. The amount of lymph in the ten minutes before the injection was 1·5 c.c. In the ten minutes after the injection, the amount was 5 c.c. After another forty minutes when the amount in ten minutes had sunk to 3 c.c. 5 decigrams more peptone were injected. In the next ten minutes after the injection 9 c.c. of lymph were obtained. In another experiment, injection of 150 c.c. defibrinated blood, to which 1·5 decigrams peptone had been added, raised the lymph-amount in ten minutes from 3 c.c. to 7 c.c. The increase thus produced is about equal to that obtained in the majority of my experiments after transfusion of peptone-blood and the question at once arises whether the blood of the dog *A* contained amounts of peptone equal to those injected in the two experiments I have just mentioned. In my earlier experiments the method of detecting peptone that I adopted, inclined me to agree with Fano, since I found the blood nearly always free from peptone. In these experiments two methods of testing for peptone were employed, similar to those used by Neumeister and Shore.

Method I. The blood was received into an equal volume of saturated solution of ammonium sulphate. The mixture was saturated by rubbing in a mortar with ammonium sulphate crystals, filtered, and the clear filtrate tested for peptone according to Neumeister's method. I found by control experiments that I could not detect less than 0·3 % peptone in the blood by this method, that is to say, I obtained a very faint biuret reaction, in the filtrate, if I had dissolved in the blood 0·3 % of Grübler's peptone. The specimen of Grübler's peptone that I used

contained a fair amount of albumoses, so that the strength of the biuret reaction obtained in the ammonium sulphate filtrate could only be regarded as an index of the total amount of 'Grübler's peptone' present, and not as representing the actual amount of this latter.

Method II. The blood was centrifugalised and the plasma or serum saturated with ammonium sulphate crystals and filtered. Control experiments showed that a biuret reaction could be obtained in the filtrate, if 0·06 % Grübler's peptone had been added to the blood.

It is evident that in the earlier experiments, when Method I. was employed and no peptone found in the blood, we might still have injected with the 150 c.c. blood 4·5 decigrams of peptone; and as a matter of fact, as will be seen in the second part of this paper, the application of another method for the separation of peptones and albumoses from the blood enables us to prove its presence in the blood a considerable time after its injection (1 to 2 hours) and that the disappearance of peptone from the blood is not nearly so rapid as has been generally supposed.

I have thus been able to show that the peptone still present in the blood from the dog *A*, ten or fifteen minutes after the injection of this substance, is quite sufficient to account for any lymphagogic effect the transfused blood may exert on the dog *B*.

We must conclude that the peptone exercises an excitatory effect on the endothelial cells, causing an increased flow of lymph, as Heidenhain suggested, and this increased flow is in no way conditioned by the change in the blood that is simultaneously effected by the injection of peptone.

I think my experiments tend also to show that the change in the coagulability of the blood is not dependent on the effect of the peptone on the endothelial cells of the blood vessels, at any rate, on their lymph-producing functions. The injection of peptone may act on the endothelial cells and increase the flow of lymph, or on the blood and destroy its spontaneous coagulability. These results are, however, independent, and do not necessarily occur together.

TABLE I.

Direct transfusion of peptonised blood from carotid artery of A into facial vein of B.

No. of exp.	Date	Wt. of dog *B*	Time after peptonization at which transfusion into *B* took place	Amount of blood transfused	Peptone in blood (?) from *A*. Coagulability (?)	Lymph per min. before transfusion (*B*)	Lymph per min. after transfusion	Post mortem appearances in dog *B*
(1)	May 30, 1892	6300 grms.	6 mins. (cannula blocked) Chief transfusion took place 20 mins. after injection	(?) Dog *A* bled to death into *B*	None (method I.) Blood of both dogs uncoagulable	0·3 c.cm.	0·4 c.cm.	normal
(2)	May 31, 1892	7250 grms.	5 minutes	120 grams.	None (method I.) Blood from both dogs uncoagulable	0·3 c.cm.	5·0 c.cm. (very bloody)	normal
(3)	June 13, 1892	8570 grms.	$2\frac{1}{2}$ minutes	About 150 grams.	None (method I.) Blood from *A* clotted slowly, quite firm in 3 hrs. Blood *B* unaltered	1·0 c.cm.	2·5 c.cm.	normal

TABLE II.

Indirect Transfusion. Dog A bled, and the blood allowed to flow from burette into facial vein B.

No. of exp.	Date	Wt. of *B*	Time elapsed from completion of peptone injection to bleeding of *A*	Amount of blood injected into *B*	Peptone in blood *A*	Lymph per min. before	Lymph per min. after injection	Post mortem appearances, *B*
(4)	June 8, 1892	8820 grs.	9 mins.	130 cm.	None (method I.) Blood from *A* & *B* fluid till evening	0·3 c.cm.	1·1 c.cm.	normal
(5)	June 14, 1892	9570 grs.	2 mins.	150 c.cm.	None (method I.) Blood from *A* clotted in 20 mins. Blood from *B* in 25 mins.	0·2 c.cm.	0·7 c.cm.	normal
(6)	June 16, 1892	?	2 mins.	130 c.cm.	None (method I.) Blood from *A* clotted in 10 mins. Blood from *B* did not clot for more than an hour	0·1 c.cm.	1·0 c.cm.	normal
(7)	June 18, 1892	9800 grs.	2 mins.	125 c.cm.	None (method I.) Blood from *A* did not clot. Blood from *B* clotted in $1\frac{1}{2}$ hrs.	0·35 c.cm.	1·0 c.cm.	normal
(8)	June 22, 1892	?	5 mins.	160 c.cm.	None (method I.) Blood *A* did not clot. Blood *B* clotted slowly	0·5 c.cm.	1·6 c.cm.	normal
(9)	June 28, 1892	8900 grs.	8 mins.	150 c.cm.	None (method I.) Blood *A* clotted in 20 mins. Blood *B* clotted in 5 mins.	0·4 c.cm.	2·0 c.cm.	normal

TABLE III.

Injection into the arterial system.

Injection of peptone blood from A into cerebral end of carotid artery of B.

No. of exp.	Date	Weight of *B*	Time elapsed from peptone injection to bleeding of *A*	Amount of blood injected	Peptone in blood *A*	Lymph per min. before	Lymph per min. after injection	Post mortem appearances of *B*
(10)	July 1, 1892	6280 grms.	10 mins.	150 c.cm.	Blood clotted in 30 mins. distinct peptone reaction (II.)	0·15 c.cm.	0·21 c.cm.	normal
			Injection by means of long catheter into thoracic aorta of B.					
(11)	July 2, 1892		16 mins.	140 c.cm.	Blood *A* did not clot, plasma gave faint peptone reaction (II.)	0·4 c.cm.	0·8 c.cm.	normal
(12)	July 4, 1892	9000 grms.	9½ mins.	150 c.cm.	Blood *A* did not clot, plasma contained about 0·2 % peptone (II.) Blood *B* also uncoagulable contained no peptone	0·35 c.cm.	0·3 c.cm.	normal
(13)	July 6, 1892	8070 grms.	2 mins.	150 c.cm.	Blood *A* uncoagulable. Plasma contained about 0·3 % peptone (II.) Blood *B* clots slowly	0·3 c.cm.	0·9 c.cm.	normal
(14)	July 8, 1892	11,420 grms.	2 mins.	150 c.cm.	Blood *A* clotted in 15 mins. Serum *A* contained fair amount (0·2 %). (Method II.)	0·3 c.cm.	0·6 c.cm.	normal

II. On the fate of peptone in the blood and lymph.

It was shown by Shore[1] that peptone shortly after its introduction into the blood, appeared in the lymph. As we have seen above that the increased lymph-flow is conditioned directly by the presence of peptone in the blood and not on any change in the blood brought about by the injection of peptone, it becomes interesting to enquire into the relative amounts of peptone in the blood and lymph at varying times after the injection. Heidenhain[2] has shown that after injection of sugar or salt, these bodies leave the blood and are transferred by the selective activity of the endothelial cells of the blood vessels into the lymph, so that finally the latter contains more sugar or salt than the blood itself. It seemed possible that this might be also the case with peptone. The questions to decide, therefore, were:—

(*a*) How long, after the injection of peptone into the vein, can this body be still detected in the blood?

(*b*) How soon does it appear in the lymph, and what are the relative amounts of peptone in blood and lymph respectively, at definite time intervals after the injection.

Plosz and Gyergyai[3] stated that they could detect peptone in the blood three hours after its injection. They also professed to find peptone in considerable quantities in the blood of the portal vein, during digestion and looked upon the liver as the chief seat of the regeneration of peptone. These authors, however, merely acidified and boiled to get rid of the coagulable proteids, before applying the biuret test—a method calculated to show the presence of peptone in the most innocent of fluids.

Schmidt-Mulheim[4] showed that after injection of peptone into the jugular vein, it could no longer be detected in the blood after a lapse of fifteen minutes.

In one of his experiments (17) 6·3 grams peptone were injected into a dog of 28 kilos.

Blood before injection contained no peptone.
„ 2 minutes after „ 0·115 % peptone.
„ 17 „ „ „ no peptone.

[1] Shore. "On the Fate of Peptone, &c." *Journal of Phys.* xi. p. 528.
[2] Heidenhain. "Zur Frage der Lymphbildung." *Pflüger's Archiv*, Bd. xlix. 1891.
[3] Plosz and Gyergyai. *Pflüger's Archiv*, x. 536.
[4] Schmidt-Mulheim. *Du Bois' Archiv*, 1880, p. 33.

In another experiment (18) 5 grams were injected into a dog of 9 kilos.

Blood 1 minute later contained 0·067 % peptone.
„ 5 „ „ „ 0·015 % „
„ 16 „ „ „ no peptone.

On investigating the blood of digesting animals, he found in one or two cases no peptone. In others he obtained the following amounts :— 0·028 %, 0·017 %, 0·022 %, 0·011 %, 0·008 %. The determinations were made with serum or diluted blood. The proteids were precipitated by heating with ferric chloride and sodium acetate, and the filtrate evaporated to a small bulk. To this the biuret test was applied—the coloured fluid thus obtained being diluted until its tint matched that obtained with a standard (1 in 3000) solution of peptone.

He ascribed the rapid disappearance of peptone from the blood after injection to some change effected by the blood itself.

Hofmeister[1] using essentially similar methods, confirmed Schmidt-Mulheim's statements as to the rapid disappearance of peptone from the blood. In two of his experiments in which large amounts of peptone were injected, no peptone could be found in the blood fifteen minutes after the end of the injection. He ascribes the disappearance, however, not to a combination taking place in the blood, but to a rapid turning out of the peptone into the tissues, and shows that a very large amount of this body is heaped up in the kidney. In one case he was able from the kidneys alone to recover 6 % of the amount of peptone injected.

Fano[2] continuing Schmidt-Mulheim's experiments and using the same methods, finds a still more rapid disappearance of peptone.

In two experiments in which 0·3 grams per kilo were injected as quickly as possible, the peptone had totally disappeared from the blood drawn 30 seconds after the injection. In another experiment peptone could be still found one minute after the injection, but had quite disappeared five minutes later. He inclines to Schmidt-Mulheim's explanation of this rapid disappearance, namely, that the peptone enters into some sort of combination in the blood.

Neumeister[3] pointed out that the methods used by Hofmeister

[1] Hofmeister. "Ueber das Schicksal des Peptons im Blute." *Zeitschr. f. phys. Chem.* 1881, p. 127.

[2] Fano. "Das Verhalten des Peptons und Tryptons gegen Blut und Lymphe." *Du Bois' Archiv*, 1881, p. 272.

[3] Neumeister. "Ueb. die Einführung der Albumosen und Peptone in den Organismus." *Zeitschr. f. Biol.* XXIV. p. 272.

and Schmidt-Mulheim were open to the objection that in the boiling with dilute acid, a certain amount of primary albumoses might be formed, and so render the test for peptone worthless. He showed that the blood of digesting animals was quite free from peptone or albumoses, so that the positive results of earlier observers were due to an error in the methods employed.

In order to detect peptone in blood, Neumeister used ammonium sulphate to precipitate all coagulable proteids and filtered. The filtrate was treated with an equal bulk of saturated caustic potash, the precipitate of potassium sulphate filtered off, and a drop of 2 % copper sulphate added to the filtrate. By this method he was able to detect 0·02 % peptone in solution. This method is, however, open to several objections

In the first place all the primary albumoses are precipitated by saturation with ammonium sulphate, so that when the liquid contains Grübler's peptone, only a certain percentage is present in the filtrate to give the reaction.

Secondly, the delicacy of the test is materially affected by the amount of coagulable proteid present. The very thick flocculent precipitate produced by ammonium sulphate carries down with it mechanically a large amount of peptone, just as is the case with animal charcoal. Thus in a watery solution it was easy to show by Neumeister's method, the presence of peptone in a fluid which only contained 0·02 % of the specimen of Grübler's peptone[1] I employed.

If plasma or serum were used instead of water to dissolve the "peptone," not less than 0·06 % could be detected, and if defibrinated blood were used, the limits of the reaction were reached at 0·3 %. Moreover I did not find that one could get much further by concentrating the filtrate from the ammonium sulphate precipitate. The fluid soon becomes a dense mass of crystals, from which only a few drops of fluid can be obtained, and it seemed probable that the peptone present must adhere to the crystals, since the concentrated filtrate appeared to contain no more peptone than the same fluid before concentration.

Thirdly, the time involved is considerable, owing to the time required to saturate the solution with the salt, and the slowness with which the fluid, free from proteids, passes through the filter paper.

[1] I may mention that Grübler's peptone is not an invariably constant preparation. Throughout these experiments I used the same specimen of peptone. This gave a distinct precipitate on saturation with ammonium sulphate, and contained a fair amount of albumoses.

It was necessary then to find some method of precipitating the coagulable proteids in blood which should fulfil the following conditions:—

(1) The separation must take place in the cold, in order to avoid the possible production of primary albumoses.

(2) The precipitate must be fine, so that it does not carry down peptones with it, and must be easily separable by filtration.

(3) The filtrate must be such as to admit of concentration by evaporation, and must at the same time allow of the immediate application of the biuret test.

These conditions are all fulfilled by the use of trichloracetic acid. This substance was recommended by Raabe[1] and by Kowalewsky[2] as a delicate test for proteids in urine. Obermayer[3] showed that this acid might be used to precipitate the total proteids present in milk, urine, exudations, &c. According to this author, trichloracetic acid produced a precipitate in a concentrated solution of peptone, which was easily soluble in excess of the reagent.

Fraenkel[4] has recently used trichloracetic acid in 3% solution, to extract glycogen from the liver. He states that the glycogen obtained by this method is perfectly free from proteids. I have found that trichloracetic acid produces no precipitate whatever in a 1% solution of Grübler's peptone. On the other hand it effects a nearly complete separation of the coagulable proteids in plasma, serum and blood.

In applying this method, 10 c.cm. of plasma are treated with the bulk of a 10% solution of trichloracetic acid, and the mixture well shaken, and then filtered. Filtration takes place extremely quickly, and the filtrate is a clear colourless liquid, almost entirely free from proteids. It gives none of the reactions for proteids. If concentrated to one-fourth its bulk on a water bath, it will then give a slight cloudiness with phosphotungstic acid, but the amount of proteid present is insufficient to give any reaction with nitric acid, or with the xanthoproteic or biuret tests. I have made a number of controls of this nature, always with the same result, so that there is no fear that any albumose reaction obtained, even after concentration to this extent, may be artificial in origin.

[1] Raabe. *Zeitschr. f. anal. Chem.* XXI. 303.

[2] Kowalewsky. *Zeitschr. f. anal. Chem.* XXIV. 551, 1885.

[3] Obermayer. *Med. Jahrbücher*, 1888, p. 375—381, and Maly's *Jahresbericht*, 1889, p. 7.

[4] Fraenkel. *Pflüger's Archiv*, Bd. LII. p. 125, 1892.

The biuret test is applied directly to the filtrate and is not interfered with at all by the presence of the acid. I have always treated 10 c.c. of filtrate with 1 c.c. of saturated caustic potash, and then added, drop by drop, a very dilute solution of copper sulphate. The quantitative determination is carried out by diluting the filtrate until the tint produced by the alkali and copper sulphate is exactly the same as that produced with a solution of Grübler's peptone of known strength. I have generally used the strength recommended by Schmidt-Mulheim, viz. 0·03%. The number of times the filtrate must be diluted in order that the tint corresponds to the standard, multiplied by $2 \times 0{\cdot}03$, gives the percentage amount of "peptone" present in the original fluid.

If a solution of peptone in plasma be made containing 0·06%, it will be found that the biuret reaction obtained in the filtrate, after separation of the coagulable proteids, is very nearly the same as that given by the standard solution (it gives actually ·04% or ·05%), showing that the amount of peptone lost by adherence to the precipitate is extremely small.

By this method, with evaporation of the filtrate to one-fourth its bulk, it is possible to detect peptone in plasma containing only ·005%.

In my actual estimations of peptone in the blood, after it has been injected into the veins, I have not found it necessary to concentrate the filtrate at all.

If concentration is necessary, it should not be allowed to go on too rapidly at a high temperature. If allowed to proceed too quickly, the fluid becomes yellow, and the application of the biuret test is rendered very difficult, if not impossible.

The experiments were carried out as follows: The animals (dogs) were anæsthetised with a large dose of morphia, and a mixture of chloroform and ether. Cannulæ were inserted into the thoracic duct, or carotid artery, and into a femoral or jugular vein. Then Grübler's peptone (0·3 or 0·5 grams per kilo) dissolved in about 25 c.c. warm salt solution was allowed to run into the vein. The time taken in the injection was about three minutes. At varying intervals after the injection, 60 c.c. of blood were drawn from an artery into a centrifuge glass. The lymph was collected in different tubes, so that each specimen corresponded to a given specimen of blood. (Thus, if blood was drawn at 20 minutes after the injection, the lymph that flowed from 17 minutes to 23 minutes after the injection was taken.)

In the afternoon the blood was centrifuged and the plasma (or

serum, if the blood had clotted) and lymph were examined for peptone in the way I have described.

The protocols of 4 experiments of this nature are given at the end of the paper. It is evident from these results that the rapidity with which peptone disappears from the blood after it has been injected has been very much exaggerated. The shortest time at which I have found total disappearance of the peptone injected was 50 minutes after the injection (Exp. 2). In Exp. 4, the blood-plasma still contained ·04% peptone 70 minutes after the injection. Ten minutes after the injection, at which time, according to Fano, all the peptone should have disappeared, the blood still contained in Exp. 1, 3, 4, 0·2%, 0·36%, and 0·21%. In fact, it cannot be maintained that peptone disappears more rapidly from the blood than any other abnormal constituent, or than any normal constituent, such as sugar, which has been introduced in large quantities.

The second point on which I wished to obtain information, viz. the relative amounts of peptone in blood-plasma and lymph respectively, after the injection of peptone into the blood, is illustrated by the tables given in Plate V. These represent graphically the amounts of peptone in blood and lymph. It will be seen from them that the behaviour of peptone after injection is almost exactly analogous with that of sugar. Thus in Exp. 3 two minutes after the completion of the injection, the plasma contained ·48% of peptone and the lymph only ·24%. In the next 10 minutes, the amount of peptone in the blood falls rapidly, while that in the lymph rises, so that 10 minutes after the injection the two amounts are the same. After this, both amounts fall gradually, the fall in the blood, however, being more rapid than that in the lymph, so that 20 minutes after the injection the peptone in the blood and lymph is ·18 and ·30 respectively, and after 50 minutes ·10 and ·18. Here then we have, as it was pointed out by Heidenhain in the case of sugar, a passage of a substance from a medium—the plasma containing less of it to a medium, the lymph containing more, a process which can only be ascribed to an active secretory intervention of the endothelial cells of the capillary wall.

In a second series of experiments (protocols 5 to 8) the renal vessels and ureters on both sides were ligatured at the commencement of the experiment, before the injection of peptone. This operative procedure makes a very slight difference in the curves obtained when sugar or salt is injected. The amount in the blood of these two bodies does not sink so rapidly; hence the lymph-curve does not cut the

blood-curve so soon as under normal conditions. The further course of the two curves moreover is rather flatter, owing to the same cause. In the case of peptone, however, the aspect of the curves obtained is completely altered if the kidneys be previously cut out of the circulation. It will be noticed that in Exp. 6 and 8, the amount of peptone in the lymph does not rise at any time higher than that in the blood-plasma, and in Exp. 7, the difference to the credit of the lymph is very small indeed.

An explanation of the difference between these two sets of experiments is not very easy to give. I thought at first that the variation might be due to the fact that the blood was unable to get rid of its surplus peptone by the kidneys and therefore the percentage amount of this substance in the blood did not fall so rapidly as under normal circumstances. This view is at once negatived by a comparison of the two sets of curves. It will be seen that in the cases with ligatured kidneys it is not the blood-curve but the lymph-curve which is altered. The peptone in the blood seems to fall as rapidly as in the first series of experiments.

I think that probably the difference is due to a varying activity of the vascular endothelial cells in the several organs of the body.

Hofmeister has shown that, after the injection of peptone, a large accumulation of peptone occurs in the kidney, although no urine whatever is secreted. If the kidney tissues become thus loaded with peptone the lymph draining away from this organ must also contain a large amount of this substance. If this view be correct the large preponderance of peptone in the lymph over that in the blood, in animals with intact kidneys, is due chiefly to the large secretion of peptone by the endothelial cells of the renal capillaries. I hope however to investigate this point further.

It will be seen from my experiments that the loss of coagulability of the blood that is produced by the injection of peptone has no connection with the amount of peptone present in the plasma. Thus in Exp. 4, the blood, 70 minutes after the injection, was uncoagulable, although the plasma only contained ·04%. On the other hand, in Exp. 6 and 8, in which the coagulability of the blood was not affected, the plasmata of the blood drawn off two minutes after the injection, contained ·36 and ·30% of peptone respectively. We may conclude that the varying effect of peptone on the coagulability of the blood is not conditioned by a varying rapidity of its excretion from the blood.

It will be noticed that in 3 out of 4 experiments in which the

kidney-vessels were ligatured, the coagulability was not affected by the peptone. My experiments, however, are not sufficient in number to decide whether this deviating result is to be referred to the operation or merely to an accidental agglomeration of immune dogs.

Summary.

1. When peptone is injected into the circulation of a living animal, it disappears gradually and may still be traced in the plasma from one to two hours after the injection.

2. The disappearance is caused in the first place by a transfer of peptone from the blood to the lymph, so that shortly after the injection the lymph contains more peptone than the blood-plasma itself. This transference is effected by the selective activity of the cells of the vessel-wall, and it is probable that a preponderating part is played by the endothelial cells of the renal capillaries.

3. There is no connection between the loss of coagulability induced by the intravascular injection of peptone and the amount of peptone present in the plasma at any given time after the injection.

My warmest thanks are due to Professor Heidenhain for the unwearying kindness and suggestive criticism with which he has helped me in this work.

PROTOCOLS. (Kidneys Free.)

Exp. I. *July* 15, 1892. *Dog* 13 *kilos.*

At 10.47 to 10.49—7 grams peptone injected.

Blood plasma fluid	10 minutes later	contains	0·2 %	Lymph	0·2 %
	15 „	„	„	0·16 „	„	0·27 „
	20 „	„	„	0·10 „	„	0·18
Partially clotted	30 „	„	„	0·08 „	„	0·06 „
	40 „	„	„	0·04 „	„	

Exp. II. *July* 19, 1892. *Dog* 10½ *kilos.*

10.37 to 10.40—5 grams peptone injected.

Blood fluid	30 minutes later	0·06 %	Lymph	0·15 %
	40 „	„	0·015 „	„	0·27 „
	20 „	„	0·10 „	„	0·10 „
Clotted	60 „	„		„	0·6 „
	80 „	„		„	0·04 „

Exp. III. *July* 20, 1892. *Dog* 12·3 *kilos.*

10.57 to 11.0—6 grams peptone injected.

Blood-plasma	After	2	minutes	0·48 %	Lymph	0·24 %
(all unclottable)	„	10	„	0·36 „	„	0·36 „
	„	20	„	0·18 „	„	0·30 „
	„	30	„	0·15 „	„	0·30 „
	„	50	„	0·10 „	„	0·18 „
Blood itself	„	60	„	0·04 „		

Exp. IV. *July* 21, 1892. *Dog* 11½ *kilos.*

10.47 to 10.50—6 grams peptone injected.

Blood-plasma (all specimens uncoagulable).

2	minutes	0·42 %	Lymph	0·12 %	
10	„	0·21 „	„	0·27 „	
20	„	0·15 „	„	0·24 „	
50	„	0·09 „	„	0·15 „	
70	„	0·04 „	„	0·09 „	

90 mins. (No blood obtainable, dog died).....(6).....0·09

PROTOCOLS. (Kidney Vessels ligatured.)

Exp. V. *July* 22, 1892. *Dog* 7 *kilos.*

Inj. 3·5 grams. peptone from 10.37 to 10.40.

Serum after	30	minutes	0·10 %	All specimens of
	50	„	0·06 „	blood clotted
	70	„	0·04 „	
	90	„	0·04 „	

2 hours a trace.

Exp. VI. *July* 25, 1892. *Dog* 8·8 *kilos.*

3 grams peptone injected from 11.12—11.15.

Blood-serum after	2	minutes	0·36 %	Lymph	a trace	All specimens
	10	„	0·18 „	„	0·12 %	of lymph and
	30	„	0·09 „	„	0·09 „	blood clot
	50	„	0·05 „	„	0·04 „	
	70	„	0·03 „			
	90	„	0·03 „	„	no lymph.	

Exp. VII. *July* 28, 1892. *Dog* 8½ *kilos.*

10.57 to 11.0—4 grams peptone injected.

Blood-plasma after	2	minutes	0·30 %	Lymph	0·09 %	Blood uncoagulable
	10	„	0·18 „	„	0·18 „	
	20	„	0·12 „	„	0·15 „	
	40	„	0·06 „	„	0·09 „	

Exp. VIII. *July* 30, 1892. *Dog* 10 *kilos.*

3·5 grams peptone injected.

Serum	2 minutes	0·30 %	Lymph	0·06 %	All specimens blood
10	„	0·15 „	„	0·12 „	clotted
20	„	0·06 „	„	0·06 „	
40	„	0·03 „	„	0·02 „	
60	„	a trace	„	a trace	
90	„	a trace	„	a trace	

DESCRIPTION OF PLATES V. VI.

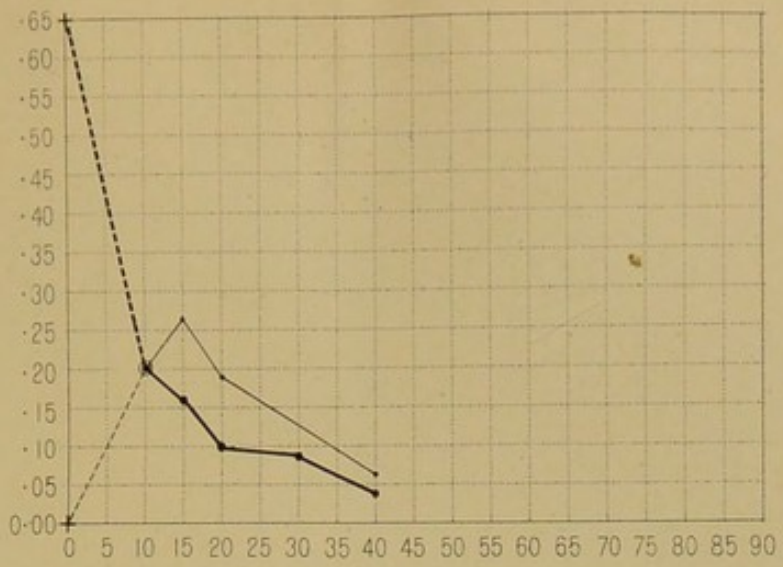
Each division of the abscissa represents a time interval of 5 minutes after the conclusion of the peptone injection.

Each division of the ordinates represents 0·05 % of peptone.

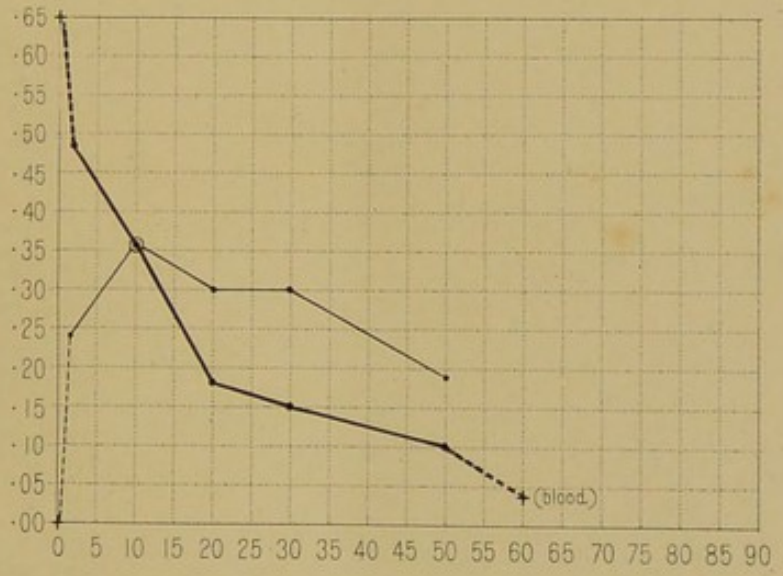
The percentage amount of peptone in the lymph is represented by the thin line, that in the blood by the thick line.

Plate V. Three experiments showing relative amounts of peptone in blood and lymph, after injection of peptone into normal dogs.

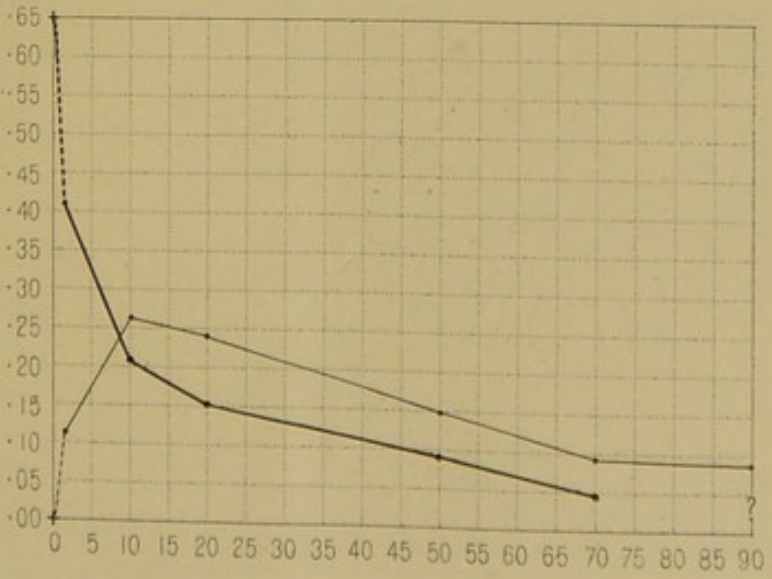
Plate VI. Three experiments showing relative amounts of peptone in blood and lymph, after injection of peptone into dogs in whom the renal vessels and ureters on both sides have been ligatured.



Exp. 1. July 15th



Exp. 3. July 20th



Exp. 4. July 21st

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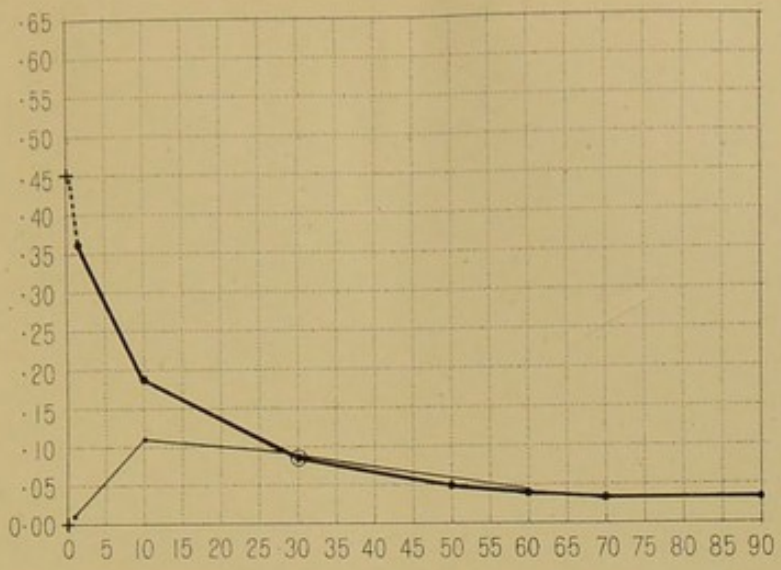
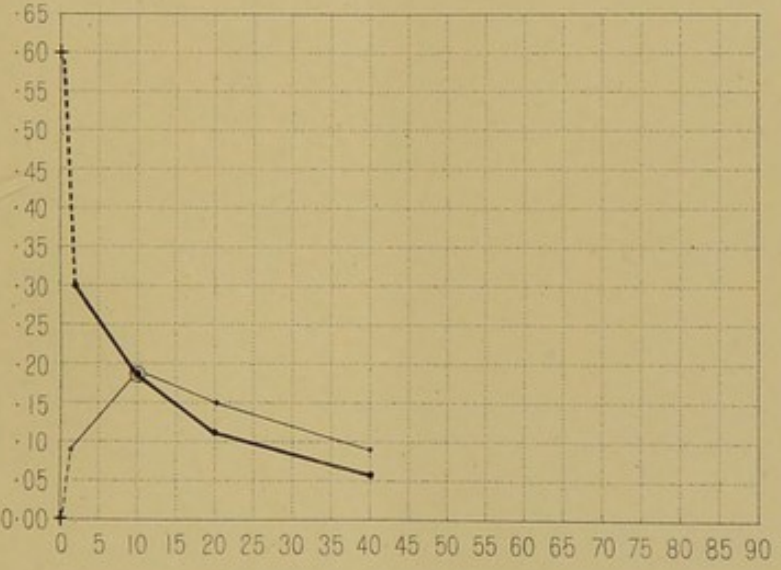
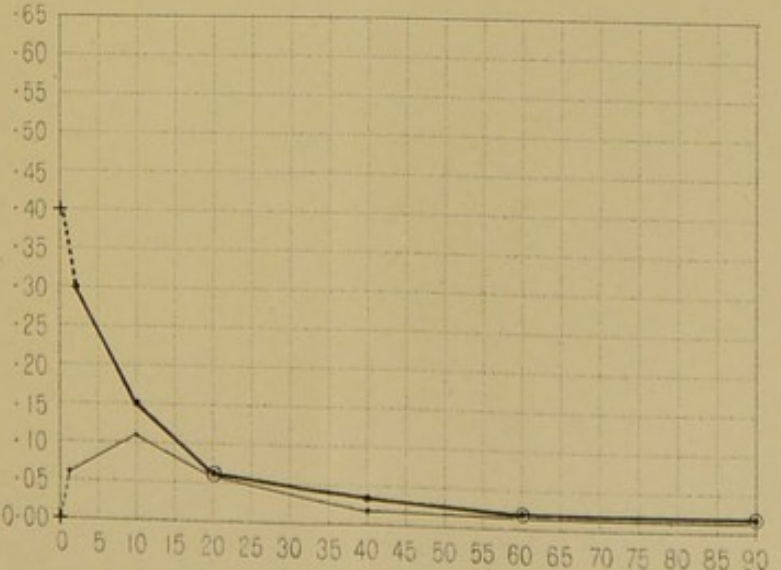


Exp. 6. July 25th



Exp. 7. July 28th



Exp. 8. July 30th

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