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CHANGES IN RETICULOENDOTHELIAL STORAGE DURING ONTOGENESIS WITH PARTICULAR REGARD TO STORAGE IN THE LIVER

(Investigations with traced bismuth trisulphide colloid)

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Introduction

There are few data concerning the changes occurring in the function of the reticuloendothelial system during ontogenesis. It is known from earlier microscopic examinations performed with vital dyes and colloids that in embryonic life many cells are endowed with a phagocytosing and colloid-storing capacity which is ascribed to the reticulo-endothelial cells (*Okkels, Beard, Hanan, Schwarz, Dellepiane, Becker* etc.). To our best knowledge, no systematic and above all no quantitative investigations have been made into this question. That reticulo-endothelial activity should not be characterized with misleading microscopic pictures but with exact quantitative data has often been urged in the literature of recent years (*Halpern et al., Jancsó et al., Törő et al., etc.*). This has prompted us to make experiments with RaE-traced bismuth trisulphide colloid in hens in the different periods of ontogenesis. The present paper gives a brief account of recently acquired data referring to changes in the storing function of the reticuloendothelial system during ontogenesis.

Some of the authors who observed the distribution of isotope or traced colloid under different conditions do not mention reticuloendothelial function, while others again bring the conditions of the distribution into correlation with the storing function of the reticuloendothelial system. In our opinion, reticulo-endothelial activity plays a decisive role in the distribution of the colloids within the organism, albeit other factors, such as haemodynamic ones, or the permeability of vessels, are also of influence. For that very reason, the conditions of colloid distribution offer valuable clues as to reticuloendothelial function.

Investigation in the embryonic stage of the ontogenesis into the function of the reticuloendothelial system involves special technical difficulties, especially in mammals.

In the course of the present investigations and when evaluating the experimental results it became clear that the difficulties are greater than expected and that the question investigated is an extremely complex and complicated one.

Hence the published results are intended to raise certain aspects of the problem rather than to establish a definite opinion. The mentioned difficulties will be dealt with in the discussion. Our results have, however, seemed worth publishing since identical data could not be found in the literature and since they might present a basis for further studies.

Material and Methods

Bismuth trisulphide colloid traced with RaE was used in the experiments. Preparation of the colloid and execution of the measurements were as follows.

From a RaD standard solution the RaE (radio-bismuth) in radioactive decay equilibrium is settled in H_2 stream onto a Pt-disc from where it is dissolved with concentrated HNO_3 and evaporated. The activity on the disc is taken up into a freshly prepared solution of about 40 mg of Bi-lactate per 100 ml, containing 0.2 per cent gelatine. Then, with continuous stirring, at $0^\circ C$, an equal volume of Na_2S solution 10 per cent in excess is added in a thin jet. Thereafter the solution is adjusted with 0.1 n NaOH to pH 7. Accordingly, the end concentration was on the average 20 mg for bismuth and 1 per cent for the gelatine stabilisator. When kept under sterile conditions the colloid showed flocculation only after 2 to 3 weeks. Its specific activity was $\sim 2.5 \mu C/ml$ of colloid.

Measurement of RaE radiation was carried out with a Geiger—Müller end-window counter tube equipment, described in an earlier paper. For measurement the material was prepared in standardized glass dishes. Smaller samples (0.1 to 0.2 g) were dried after dissolution in concentrated HNO_3 and a few drops of perchloric acid. More voluminous material was concentrated either by incineration or by digestion with nitric acid and sulphuric acid. If necessary, the self-absorption of the sample was also taken into account.

The experiments were carried out in chick embryos of different ages, in one-day chicks and in hens. Embryos and chicks of the identical strain were used in each series of experiments.

In chick embryos the colloid was injected into the chorioallantoic vein. A mediumsized chorioallantoic vein and the direction of the blood stream were marked over a lamp on the shell of eggs incubated for various periods. At the marked area a few mm of the shell were removed above the vein, taking care not to injure the shell membrane. After making the shell membrane transparent by means of paraffine oil, the vein running underneath it became visible. The colloid was injected in the direction of the blood stream. A tuberculin syringe was connected by a thin rubber tube with a 0.1 ml pipette bearing a 0.001 ml graduation, and to the tapering end of the pipette a fine hypodermic needle was adjusted with a rubber tube. After the needle had been introduced into the vein the injection was performed by carefully turning the piston of the tuberculin syringe. In this way it was possible to perform the injection with a precision of 0.001 ml and to estimate 0.0001 ml. When extracting the cannula, some fluid always escapes. This reduced the precision of the injection to 0.001 to 0.002 ml. With 30 mg of bismuth colloid per 100 ml, this corresponds to about a precision of 0.2 μg . The cannula was extracted from the vein only after the colloid had been obviously carried off by the blood stream. At extraction haemorrhage is bound to arise. It was found that this extravasated blood, the adjacent shell membrane, and the chorioallantois may contain 1 to 15 per cent, in some cases even 20 per cent, of the injected activity. Since this amount had not passed into the embryo, the percentage of the colloid content of the single organs was computed by subtracting this amount from the injected one.

The amount of colloid injected as indicated in the Tables always means the difference between the colloid injected and the amount that remained at the site of the injection.

After injection the eggs were placed back into the incubator, then at the time set, mostly two hours following the injection, the embryos were removed from the eggs, exsanguinated through the umbilical vessels and dissected. This met with great difficulties, especially in the case of young embryos considering that embryonic organs are extremely soft and loose. Removed organs may contaminate each other and this can change the activity values.

From among the abdominal organs the liver is the easiest to isolate. The colloid storing capacity of the liver is great, hence the slight contamination by the adjacent organs is negligible. Quite different is however the position with the contaminating effect of parts that become detached when excising the liver. If, for instance, a small liver particle remains attached to the digestive tract, it may, owing to its high colloid content, greatly alter the true values. Owing to the aforesaid difficulties the wet weight of the organs was measured in part of the experiments

only. In the rest the embryos were fixed before dissection in Carnoy's fluid. (In general, the brain, eyes, liver, heart, spleen, digestive tract, lungs, urogenital apparatus, trunk, limbs, head and neck were isolated. In quite immature embryos the spleen was not examined separately. In some cases the activity of the chorioallantois, of the yolk sac and of the amnion fluid was also estimated.)

One-day old chicks were injected with the colloid into the jugular vein, after having made a small incision on the skin of the neck without anaesthesia. The chicks were exsanguinated two hours later and dissected into organs.

With hens the injection was given into the wing vein and they were exsanguinated two hours later. In order to ascertain the speed of the colloid's disappearance from the blood, blood samples were repeatedly taken from the prepared carotid artery by means of always fresh glass cannulas in one hen. Chick embryos were injected with an activity of about 0,1, to 0,4 μ C in 0,05 to 0,1 ml of colloid; into one-day old chicks activity of about 0,5 to 1,0 μ C in 0,5 ml of colloid; and into hens an activity of about 2 to 4 μ C was injected in 1,0 ml of colloid.

Experimental results

The main purpose of the experiments was to study the changes occurring in the storing capacity of the liver. Therefore, in the first series of experiments liver storage was examined at different intervals following the injection. According to the results shown in Table I and Fig. 1, storage in the liver attains its maximum at about 2 hours after the injection, to decrease from then on. The colloid content of the entire embryo is the highest similarly at about the 2nd

Table I

Variations in the colloid content of the liver and of the embryonic parts minus liver in 11- and 17-day embryos, following injection of colloid

Embryo		Wet weight of liver in μ g	Amount of colloid injected into the embryo in μ	Time elapsed between injection and killing	Amount of colloid stored in the liver in μ g	Storage in liver in percents	Per-centile storage of liver per 100 mg	Total storage of embryonic parts minus liver in μ g	Total storage of embryonic parts minus liver in %	Amount of colloid recovered in the embryo in μ g
age	weight in g									
11	4,10	69,0	6,24	0h15'	0,61	9,82	14,23	0,40	6,42	1,17
11	3,30	52,0	6,00	1h00'	0,95	15,98	30,73	0,68	11,47	2,18
11	3,81	70,0	4,88	2h00'	1,09	22,34	31,91	1,41	28,97	4,03
11	2,50	37,0	9,89	6h00'	0,97	9,84	26,60	1,05	10,62	2,19
11	4,60	99,0	5,13	15h52'	0,69	13,52	13,65	1,29	25,25	3,39
17	15,95	383,0	9,46	0h28'	1,48	15,70	4,10	1,39	14,76	3,48
17	15,45	352,0	9,43	1h10'	2,18	23,14	6,57	0,99	10,57	4,00
17	15,60	434,0	8,72	2h10'	3,49	40,03	9,22	1,21	13,96	6,04
17	18,30	434,0	9,00	3h10'	2,45	27,21	6,26	1,42	15,82	4,73
17	17,80	456,0	10,01	4h00'	3,70	37,00	8,11	1,08	10,81	4,83
17	17,65	432,0	9,66	6h20'	2,61	27,04	6,25	1,43	14,90	4,45
17	21,25	528,0	9,40	22h05'	2,37	28,21	5,34	1,06	12,69	3,89

hour (as to absolute quantity as well as in comparison with the amount injected). This shows that the distribution of the colloid changes rapidly between the embryo and the extraembryonic parts. The liver seems to play an important role in this changes because it was found that the excreting capacity of the liver

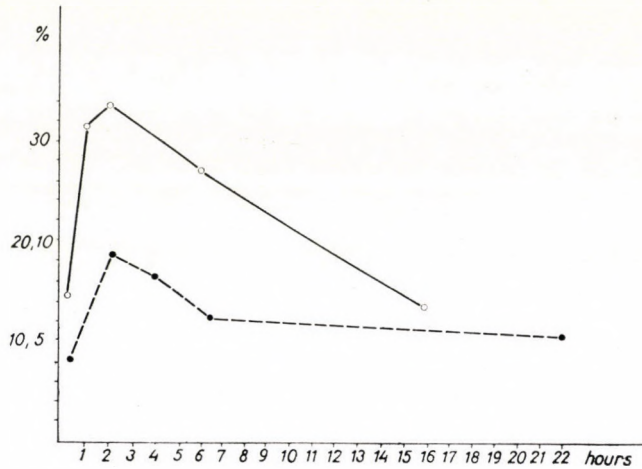


Fig. 1. Variations in time of liver storage after injection in 11 (—) and 17-day old (---) chick embryos. Ordinate: Percentage of injected activity (per 100 mg of wet liver)
Abscissa, time elapsed since injection

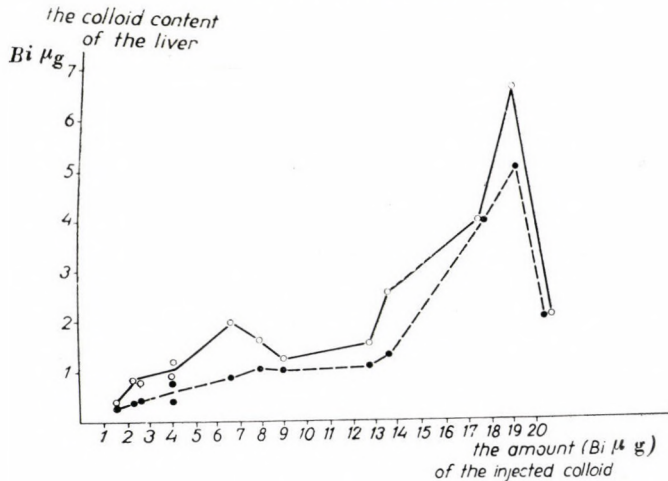


Fig. 2. Correlation of colloid injected and the amount stored in the liver

is rapid and considerable, in agreement with our previous observations made with vital dyes and India ink. In further experiments the embryos, as well as the chicks and the hens, were killed in the 2nd hour, since, as judged from the disappearance of the colloid from the blood, storing processes are completed by the

2nd hour (Fig. 3). In adult animals, excretion of the colloid stored in the liver is a slower process.

In adult animals the correlation between the amount of colloid injected and that stored in the liver was within certain limits a direct one. (*Törő, Barka, Aros, Velősy.*) This question was studied also in 11-day old embryos. In spite of the scattering experimental results it could unequivocally be ascertained that by increasing the amount of the injected colloid the quantity stored in the liver increases too. In 11-day old embryos the amount of colloid stored in the liver increases up to the injection of about 20 mg of bismuth when the bismuth stored in the liver amounts to about $7\mu\text{g}$ (Fig. 2). It was not determined what heights the blood colloid level attains 2 hours after various amounts of colloid have been injected. It might occur, particularly after injecting a large amount of colloid, that such an increase of the blood level will ensue that, considering the high blood content of the liver, the true storage value will be raised. In our opinion, however, this will not disguise the aforesaid correlation.

The distribution of the bismuth colloid was investigated in two groups of embryos of different age. In the first group the embryos were fixed in Carnoy's solution before dissection. In the second group, made up of 11- and 18-day old chicks, the wet weight of the organs was also measured. The experimental results are presented in Tables II and III.

Table II

Distribution of traced bismuth trisulphide colloid in the organs of chick embryos of different ages, 2 hours following the injection of colloid

Age of the embryo	Amount of colloid injected into the embryo in μg	Colloid content of the organs of the embryo in per cents										Colloid content of foetal membranes μg	Amount of recovered colloid in per cent
		liver	eyes	brain	head, neck	heart	spleen	digestive tract	lungs	urogenital apparatus	trunk and limbs		
8	7,83	2,5	0,3	0,25	2,1	0,47		0,24	0,13	0,8	1,4	0,49	19,4
9	6,87	7,8	0,6	0,36	2,8	0,40		0,40	0,20	1,1	3,2	1,63	39,4
9	8,19	6,5	0,4	0,6	1,1	0,30		0,10	0,10	0,8	1,6	1,14	26,3
10	6,50	9,3	0,6	0,3	2,0	0,50		0,30	0,20	1,2	5,5	1,87	51,1
10	5,96	8,0	0,6	0,4	2,2	0,30		1,00	0,60	1,0	2,2	3,05	76,2
10	5,45	10,6	2,2	0,9	0,8	0,70		0,90	0,30	3,4	6,5	0,62	48,6
10	5,74	11,7	1,3	1,8	2,9	0,30		0,60	0,30	4,4	3,9	1,41	56,3
14	12,15	33,4	0,5	0,5	4,7	0,50	0,5	0,40	0,30	—	6,3	3,95	81,6
15	6,03	31,1	0,8	0,6	5,7	0,60	0,3	0,90	0,30	1,8	7,4	0,77	66,2
17	7,80	33,3	0,4	0,2	5,1	0,2	0,9	0,40	0,30	1,0	6,9	1,87	73,0
19	7,67	51,8	—	0,4	2,6	0,3	0,5	0,80	0,25	0,6	2,3	1,19	75,3
19	8,34	65,0	0,4	0,3	6,5	0,6	1,3	1,2	0,50	0,8	2,9	0,58	86,5

Table III

Distribution of traced bismuth trisulphide colloid in the organs of 11- and 18-day old chick embryos, 2 hours following the injection of colloid

Age of the embryo (day)	Amount of colloid injected into embryo, in μg	Colloid content of the embryo in per cents										
		chorio-allantois	liver	eyes	brain	neck	heart	spleen	digestive tract	lungs	uro-genital apparatus	other organs
18	19,76	2,63	20,54	0,26	0,11	—	0,13	0,53	1,1	0,34	1,30	—
18	19,91	2,82	17,88	0,18	0,08	—	0,15	0,82	0,5	0,43	1,48	—
18	19,49	6,18	13,66	0,17	0,06	—	0,09	0,44	0,99	0,21	0,42	—
18	19,56	2,12	23,46	0,14	0,07	—	0,14	0,7	0,65	0,19	0,69	—
11	8,32	1,05	8,57	0,66	0,33	0,75	0,16	—	0,26	1,14	1,47	8,69
11	8,82	8,78	2,64	0,53	0,84	1,07	0,45	0,02	0,20	0,12	0,62	4,04
11	9,38	7,45	0,83	—	0,05	—	0,15	—	0,05	0,07	0,11	1,58
11	8,90	4,62	4,69	—	0,19	—	0,26	—	0,18	0,15	0,90	—
11	8,53	2,988	7,16	—	0,22	—	0,33	0,19	0,21	0,18	1,08	—

All the 8 to 19 days old embryos but one were given 5,4 to 8 μg of colloid. From the experimental results it was learned that solely the storage of the liver increased gradually. Of the injected 7,8 μg of colloid the 8 day old embryo is able to store 0,2 μg in the liver, while the 19-day old embryo of 7,6 μg of colloid, thus from an approximately identical amount is able to store 3,8 μg , viz. about 20 times more than the younger one. Since during the period of 8 to 19 days the weight of the liver increases to about fifty times its initial value, storage computed to weight unit decreases. A considerable amount of colloid is contained in addition in the urogenital apparatus, the trunk, the limbs, as well as in the head and the neck. Apart from the liver, no regular changes could be observed in the storage of other organs. The fact must by no means be left out of consideration that the organs in question are considerably growing over the investigated embryonic period, hence the amount of the colloid stored evidently decreases in relation to weight. It is well-known that with ontogenesis and parallel with differentiation certain cells lose their phagocytosing and storing capacity. The eyes and the brain also displayed some low activity. The quantities contained, especially in the eyes, were however, so small that they might have been due to contamination by blood, amniotic fluid, etc. As shown in the Table, merely a fraction of the injected colloid was recovered in the embryonic organs worked up. According to some tentative examinations, a considerable portion of the colloid was stored in the extraembryonic parts, partly in the yolk sac and partly in the chorionallantois. Some of the colloid was found also in the amniotic fluid (in

certain cases the amnion fluid contained 0,5 to 3 per cent of the colloid). It was not examined whether the colloid enters the yolk sac or is stored only in its wall. Parallel with the growth of the embryo the amount of colloid recovered from it (19 to 86 per cent) also increased.

Essentially similar findings were yielded by the embryos presented in Table III. In these, too, storage was the most considerable in the liver and the urogenital apparatus, while in 18-day old embryos the colloid content of the lungs was also remarkable. Comparing the values to weight, the most intensive storage occurred in the spleen, the liver and the mesonephros. Although the highest storage per weight was observed in the spleen, on account of the low weight of that organ its colloid content is negligible in the distribution of the whole amount of injected colloid. It is seen in the Tables that the divergence of parallel experiments was marked. We shall come back to the causes of this in the discussion. Still greater was the discrepancy if the amount of the colloid was computed to weight. These values are not shown in the Tables.

Table IV

Distribution of traced bismuth trisulphide colloid in the organs of 1-day old chicks and hens, 2 hours following injection of colloid

Amount of injected colloid in		Percentage of the colloid content of organs										
		liver	brain	heart	spleen	lungs	kidney	thymus	bone-marrow	bile + gall bladder	blood of 2 hours	yolk-sac
One day old chick	21,9	55,11	0,13	0,28	1,47	0,5	2,05	—	—	0,03	1,58	1,54
	21,9	63,40	0,05	0,22	1,02	0,45	1,98	—	—	0,04	1,02	1,76
	21,9	68,64	0,05	0,19	1,61	0,53	2,76	—	—	0,09	1,01	0,72
	21,9	62,04	0,07	0,27	2,46	1,52	2,47	—	—	0,07	0,97	—
	21,9	51,53	0,09	0,50	1,84	0,59	4,62	—	—	0,14	1,56	1,03
Mean	60,15	0,08	0,29	1,68	0,72	2,78	—	—	0,04	1,23	1,26
hen	242,32	56,44	—	0,03	0,62	0,19	3,76	0,01	0,06	0,05	0,07	—
	228,20	66,70	—	0,02	0,56	0,19	9,58	0,07	0,09	0,02	0,03	—
	130,20	72,20	—	0,03	0,39	0,19	3,35	0,03	0,07	0,03	0,03	—

For the sake of comparison the distribution of the colloid was studied also in one-day old chicks and in hens. In Table IV it is shown that, both in the one-day old chick and in the hen, about 60 per cent of the injected amount of colloid is stored in the liver. In 18-day old embryos liver storage shows about the same rate. Both in one-day old chicks and in hens the storage rate proceeds in the order liver, kidney, spleen, lungs. In one-day old chicks the unresorbed yolk-sack wall contains a considerable amount of colloid, approximately the amount

stored in the spleen or in one kidney. In the embryo as well as in the one-day old chick and in the hen the bile contained colloid as early as in two hours, a sign indicative of prompt excretion. In a hen the speed of disappearance from the blood of the colloid was determined in blood samples taken serially from the carotis artery. The findings were similar to those previously observed in rabbits, showing the disappearance of metal colloid from the blood (Fig. 3). (Barka, Pósalaki, Kertész.)

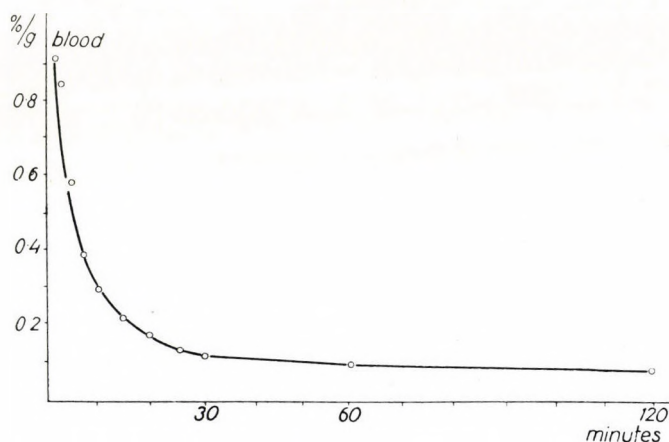


Fig. 3. Disappearance from the blood of intravenously administered BiS_3 -colloid in a hen. Ordinate: percentage of colloid in 1 g of blood as compared with the amount of colloid injected. Abscissa, time (minutes), taking of blood after the injection

Discussion

First of all, the possibilities of evaluating the findings and the conditions of the great divergences will be discussed. As already mentioned in the introduction, studying embryonic reticuloendothelial function involves great difficulties. Some of these are inevitable and lead to a wide scattering in the results. The foremost of these are as follows.

a) Considerable differences exist between the state of development and the weight of the embryos, even if the eggs have originated from the same strain. Since the state of development of the embryo cannot be known in advance, the amount of colloid to be administered cannot be computed per weight.

b) There are considerable differences in the state of development of the extraembryonic parts. Since a considerable part of the colloid is stored in the foetal membranes it is only natural that its distribution is influenced by the state of development of the extraembryonic parts.

c) As already mentioned, various quantities of colloid may remain at the site of the injection. These quantities cannot be controlled, so that the amounts

of colloid actually passing into the embryos are different even if the amounts injected have been identical.

d) The already mentioned difficulties met with when dissecting the embryo into organs. (Loss of material, contamination).

e) The inaccuracy of weighing, due above all to desiccation during microscopical preparation. This loss may amount to as much as 20 per cent.

f) Errors occurring during injection, incineration, transferring, radioactive measurements. In our opinion, these factors constitute the smaller part of the errors.

The factors just mentioned are the cause of the great divergences observed in parallel experiments. Part of the divergences can be compensated with an unusually high number of experiments. Further experiments are needed in order to reduce the sources of error.

It could unequivocally be ascertained from the experiments that in embryonic life, too, the liver is the principal storing organ beside the foetal membranes. 17—19-day old embryos, one-day old chicks as well as hens store in the liver, an almost identical fraction, about 60 per cent of the injected colloid. In earlier experiments it was already observed in rats and rabbits that, within a fairly wide range, the amount of colloid stored in the liver always to the same percentage of the quantity injected. It is thought that this percentage depends on the character of the metal colloid injected, above all on its dispersity, as well as on the activity of the reticuloendothelial system of the animal in question. In experiments performed with radioactive chromium phosphate, *Gabrielli* reached a similar conclusion. From the present experiments it appears that the same characteristic distribution of the colloid stored occurs also in the hen. This characteristic distribution dates accordingly from as early as the end of the embryonic life.

The storing capacity of other organs is negligible in comparison with the liver. If, however, storage is computed according to weight, the spleen, the mesonephros, and the lungs are also capable of accumulating a considerable amount of colloid.

When evaluating the present experiments it must be taken into account that the distribution of a colloid depends not only on the activity of the reticuloendothelial system but is also influenced by permeability conditions. Permeability is a particularly important factor in the distribution of colloid in embryonic organs, such as the brain. It is only natural that if, for instance, the brain contains colloid, this may just as well be due to the permeability of the cerebral vessels than to the activity of the reticuloendothelial system.

The experiments reported were undertaken with the purpose of collecting data on the changes in reticuloendothelial activity during ontogenesis. It must be pointed out that there is no generally applied and accepted index by means of which reticuloendothelial activity could be characterized. Therefore the

problem is difficult to formulate. According to *Törő* et al., the reticuloendothelial apparatus cannot be considered uniform and its parts built into the single organs are endowed with special functions. Consequently, their function cannot be characterized by the same factor.

In order to ascertain the changes occurring in the reticuloendothelial activity of the liver during ontogenesis, the following experiments could be undertaken.

(i) Subsequent to the injection of a medium amount of colloid (determined in a preliminary experiment) the variations in time of liver storage should be investigated, viz. the point of time when the amount stored reaches its maximum.

(ii) The amount of colloid saturating the reticuloendothelial system of the liver without any severe toxic effect at the time of maximum storage should be determined, viz. the maximum amount of colloid that the liver is capable of storing.

(iii) The distribution of the colloid should be determined after half the dose saturating the reticuloendothelial system, with special regard to the investigated organs, in the present case the reticuloendothelial system of the liver, so that the fraction of the colloid stored in the liver should be established.

(iv) The activity of the whole reticuloendothelial system should be determined on the basis of the colloid's disappearance from the blood. The experiment should be carried out with half the amount of the colloid saturating the liver.

The above four experiments performed in different periods of the ontogenesis would make it possible to characterize the variations in the storing functions.

Summary

RaE traced bismuth trisulphide colloid was prepared and its distribution was examined in the chick embryo subsequently to injection into the chorioallantoic vein. It was determined that both in 11-day and 17-day old embryos storage in the liver reaches its maximum in 2 hours. The amount of colloid stored in the liver is, within certain limits, the function of the amount of colloid injected. During ontogenesis it is only in the liver that the colloid storing capacity increases significantly, in the other organs no systematical change was observed. In 18-day old embryos and adult animals storage of colloid is similar. The results attained and the difficulties of quantitative investigation into embryonic function are discussed.

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ИССЛЕДОВАНИЕ ИЗМЕНЕНИЙ НАКОПЛЕНИЯ РЕТИКУЛО-ЭНДОТЕЛИАЛЬНОГО АППАРАТА ВО ВРЕМЯ ОНТОГЕНЕЗА, С ОСОБЫМ УЧЕТОМ НАКОПЛЕНИЯ ПЕЧЕНИ

(Исследования меченым коллоидом трехсернистого висмута)

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Авторы произвели коллоид трехсернистого висмута, меченый при помощи RaE и исследовали распределение этого коллоида в зародыше цыплят после его впрыскивания в хориоаллантоидную вену. Они установили, что накопление печени достигает своего максимума 2 часа после впрыскивания, как у 11 дневного, так и у 17 дневного зародыша. Количество накопленного коллоида является в известных пределах функцией количества впрыскиваемого коллоида.

Во время онтогенеза значительно повышается только способность печени к накоплению коллоидов, у остальных органов не было установлено закономерного изменения. В случае 18 дневных зародышей и взрослых животных наблюдается подобное накопление коллоидов.

Авторы подробно излагают свои результаты и трудности при количественных ретикуло-эндотелиальных исследованиях у зародышах.

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