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DISTRIBUTION OF TRACED LEAD SALT COLLOID IN THE RETICULOENDOTHELIAL ORGANS

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The purpose of this communication is briefly to summarize our observ ations regarding of distribution traced lead sulphide colloid and its disappearance from the blood. The experiments are in close connection with investigations into the function of the reticuloendothelial system conducted for several years in our institute.

Traced or isotope colloids are especially suitable for investigations regarding the disappearance of colloid from the blood and its distribution in the organs. With their help the distribution of diminutive amounts of colloid may be measured in the organism of the animals.

Various investigations have dealt with isotope or traced colloids *(Hahn* and *Sheppard*, *Sheppard and Hahn*, *Müller* and *Rossier Hahn*, et al., *Dobson* et al., eth.*)* Among these there are, however,no data concerning colloidal lead.

Material and methods. Matured healthy female rabbits of from 2 to 3 kg body weight were used for the experiments. The animals were given into an ear vein 2 mg per kg body weight colloidal lead sulphide from a solution containing 1 mg PbS per ml. After injecting the colloid and incising the marginal vein, 1 to 2 ml blood was taken from the opposite ear in fixed intervals for 3 hours. 3 hours later the animals were killed by stunning at the nape and bleeding out. The organs and parts prepared for radioactive measurements were weighed wet.

Preparation of traced lead sulphide colloid. The lead sulphide colloid used for the experiments was prepared in a standard manner from traced lead acetate, ammonium sulphide and gum-acacia as a stabilizator. The pH of the colloid was 7.6 at the time of the injection. For tracing, a radioactive isotope of lead, ThB, was used. This has a half period of 10,6 hours the system number 82, i. e. one identical with that of lead, and an atomic weight 212.

The preparation of traced lead acetate was carried out in the following way. A gold disk 8 mm in diameter was placed in Thorium emanation over an emanating Radiothorium preparation of O. Hahn, for about 24 to 48 hours. The disk was charged to a potential of —200 V as compared to the container. By means of that well known method of radioactive preparation, the actually positively charged ThA atoms, arising from the decay of emanation are collected by the electrostatic field into the gold disk. The greatest intensity of the ThB preparation was practically reached within 24 to 48 hours (radioactive equilibrum). The radioactive deposit was disolved from the gold disk with warm acetic acid and the acid was subsequently evaporated in a dish. The ThB activity remaining in the dish was taken up by the 1 per cent solution of lead acetat used for the preparation of the colloid. Standardization of the traced lead sulphide colloid thus prepared was carried out by withdrawing a sample with a micropipette of known volume, dehydrated in the glass receptacle of the Geiger-Müller counter, and determining its radioactivity expressed in units of impulse per minute. This was the standard to which according to their activity the PbS content of organs was compared.

The technique of radioactive measurements. In order to determine their activity the organs and blood samples were first digested wet. The samples not exceeding 0,5 g in weight were digested in microtubes by means of perchloric acid and concentrated nitric acid. The digested material was transferred into a standardized glass container and dried. Drying was carried out by means of an infrared drying apparatus which made it possible to avoid an eventual excrepitational loss of material. After adding some drops of distilled water to the material dried in the dish this was covered by a disk made of filter paper, in order to assure equal spreading, then the material was dried again. Selfabsorption of the material thus preparad is negligible.

The measuring equipment, devised by Prof. S. Szalay, was constructed in the Institute of Experimental Physics of Debrecen University. The equipment consisted of a special end-window Geiger—Müller countertube and of the adjustments required.

Results and Discussion

1. *Distribution of colloid in the organs.* (See Table 1.) The Table shows the lead sulphide content of the organs in gamma, calculated for 100 mg wet weight, as well as the percentage of the 2 mg/Kg of colloid injected, stored in the different organs. The amount of 2 mg per Kg body weight of lead sulphide was calculated from the quantity of lead acetate used for preparation of the colloid. The lead sulphide content of each organ was estimated by comparing its radiation to the standard.

As shown in the Table, among the examined organs of the reticuloendothelial system, the greatest affinity to colloidal lead sulphide was found in the spleen, the bone marrow, liver, kidney, lungs and adrenals, as computed for wet weight. Only in two animals was there a measurable amount of colloid in the lymph nodes while there was none in the pitutary and the thymus. (The sign »∅« in the Table means that the lead salt content of the organ was under the limit of measurability, while »—« indicated that no measurements were performed.)

3 hours after injection, the liver was found to contain an average of 27,0 per cent of the 2 mg/kg body weight of lead sulphide colloid injected, while the kidney contained. 1,3 per cent., the spleen 0,78, and the lungs 0,57 per cent. As the exact weight of the bone marrow cannot be determined, the percentage of storage could not be estimated. Generally, the total weight of the bone marrow is considered to be equal to that of the liver. Owing to the fact that, as calculated for 100 mg wet weight, the bone marrow was capable of storing twice the amount found in the liver, it is obvious that the major part of the colloid is taken up by the marrow.

The values of animal No. 2, differing widely from other data in the Table, are ascribed to the pregnancy of the animal.

We refrain from discussing the observation as no similar experiments have been carried out.

From among the organs examined the liver showed the most constant values of storage. The fluctuations of storage in the spleen, bone marrow and other organs were more varying. This has already been observed in our earlier experiments with colloidal silver. The probable reason for the variation is that the weight of the spleen is fluctuating to a greater extent than the weight of other organs. The fluctuation in weight is generally about 15 per cent, while that of the spleen may amount to 30 per cent *(Norton and Wolfe.)* The relation existing between the weight of the organs and their storage capacity has been indicated our earlier results *(Törő, Barka, Aros, Velőssy).*

The results of the present experiments are in complete agreement with literary data. The distribution of a certain metal colloid is probably under the regulation of several circumstances not yet precisely understood. In any case, the species of animal used is essential and results obtained on a certain kind of animal may not be valid for other species or for humans. Further, the distribution of a metal colloid may decisively affected by its dispersity. The average size of the colloid particles used by us is, as measured with the electron microscope, at about 200 A. From both our investigations and the experiments of other authors *(Dobson et al, Sheppard* et al.) the conclusion may be drawn that colloids of great dispersity are taken up in a high percentage by the bone marrow and the spleen and to a lesser extent by the reticuloendothelial cells of the liver. As far as the size of particles is concerned, the colloid used by us may be considered of high dispersity. The metal colloid prepared by us, as well as other preparations contain particles of different size in a certain characteristic ratio. It may be stated that organotropy determined by a certain particle size does not really exist. This means that for instance the liver may take up mostly but not exclusively, large particles. Therefore, the relation between dispersity and organotropy is a complicated problem which ought to be investigated exactly and in detail, and which has some practical importance, offering the possibility of isolated therapeutical irradiation of a certain organ by means of radioactive colloids. Some attempts in this direction have already been made.

It was surprising to note that the lymph nodes primarily noted as part of the reticuloendothelial system, practicaly did not participate in storing the intravenously injected metal colloid.

2. *The disappearance of lead sulphide colloid from the blood.* During the three hours following the injection, blood was repeatedly withdrawn from the animals, generally 8 times. The blood samples were digested and dried, their activity was measured, and from the result the PbS content of 1 ml of blood computed. The values obtained were plotted in a coordinate system as a function of time. (Fig. 1.) According to the figure, the curves of disappearance obtained by this method were greatly divergent, although the colloid had been given in the same dose according to body weight, and under similar circumstances.

The conclusion may be drawn from the finding that the disappearance rate of colloids from the circulation must be used for measuring the activity of the reticuloendothelial system only with certain precautions. Besides, mathematical evaluation of the results seems indispensable. One of the aims of our mathematical analyses was to evaluate the results of experiments of this kind.

A further aim of our analysis was to gain some information concerning the mechanism of the disappearance of the colloid from the blood.

It is remarkable that both the curve obtained and the equation derived may be applied only after mixing has been accomplished, i. e. from the moment the colloid has reached its maximum concentration.

It was endeavoured to approximate the experimental data with a function having the smallest standard deviation from the experimental findings and offering, at the same time, the best mean value. Determination of such a fun-

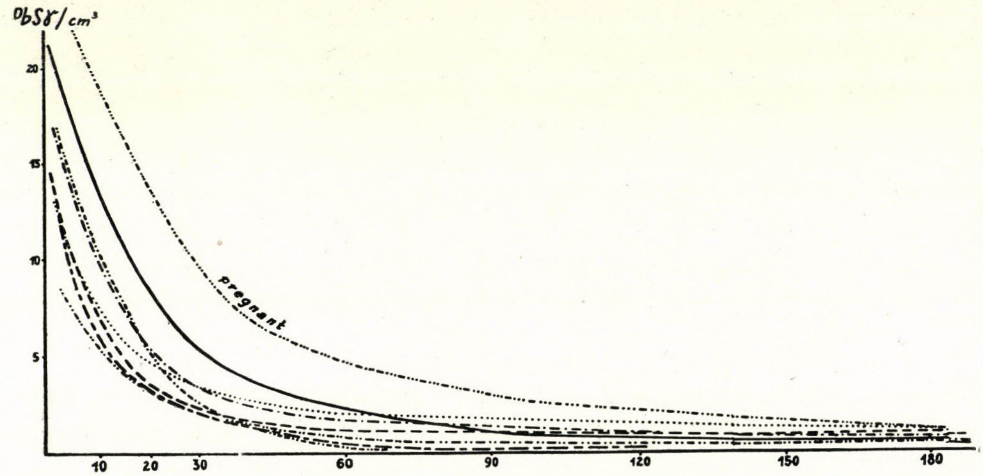

Fig. 1
The disappearance-curves of the colloid from the circulation

ction is the more important since it is indispensable for calculating the significant differences between the experimental and control groups.

The approximation was performed according to the principle of the smallest squares, with the aid of first and second order polinoms, linear fractional function (hyperbole), and an exponential function, the exponent of which consists of a polinom of the 1st or 2nd order. The functions thus obtained were plotted in Fig. 2. According to our calculations, the exponential curve seems to be the most adequate for the purpose.

A comparison with the experimental results of the exponential curves obtained showed that a closer approximation can be attained by dividing the interval under test into two parts, and performing the regression by applying an exponential function to the first phase and a lineal one to the second.

It should be noted that the limit of the intervals after division into two phases is mathematically arbitrary and has been carried out after considering the original curves.

By this method the exponential approximation of the first 15 minutes was computed as well as the lineal one of the remaining values. The calculations were repeated by taking 30 and 60 minutes for the limit.

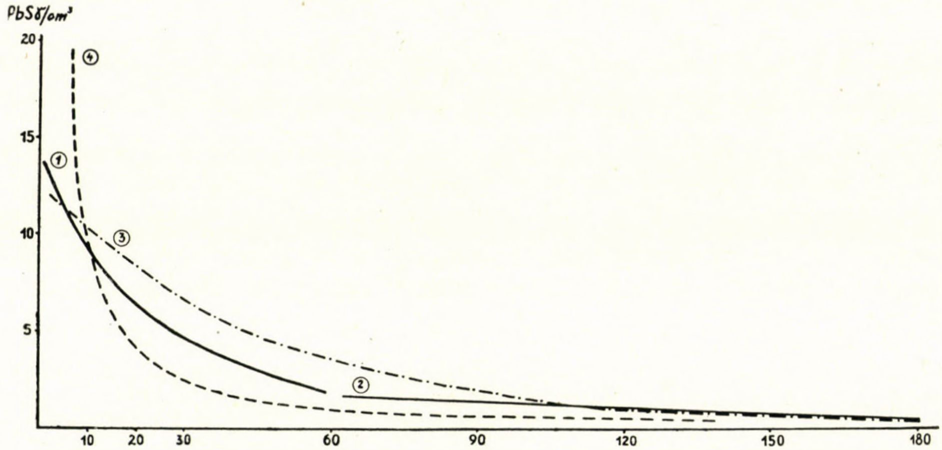

Fig. 2
Regression of the values of colloid-disappearence from the blood

1. The exponential regression of the first 60 minutes. The equation of the curve is:

$$y = e^{-0{\cdot}039306x + 2.634644}$$

2. The lineal approximation of the values measured between the 60 and 180 minutes. The equation of the curve is

$$y = -0.0118X + 2.50100$$

3. The exponential regression of the values measured between the 1 and 180 minutes. The equation of the curve is

$$y = e^{-0{\cdot}02167\ x + 2.53959}$$

4. The hyperbolic regression of the values measured beetwen the 1 and 180 minutes. The equaation of the curve is:

$$y = \frac{1}{0.0170X - 0.0707}$$

According to the analysis the double curve obtained with the exponential regression of the first 60 minutes and with the lineal one of the remaining period complies best with the real values. The equations of the mentioned curves are as follows :

$$y = e^{-0{,}039306\ X\ +\ 2{,}634644}$$

and

$$y = -0{,}0118\ X + 2.50100$$

Such curves are not only mathematically the most adequate among the calculated curves, but obviously they are in best accordance with the process.

According to the above, for drawing conclusions from curves displaying the dissappearance of a colloid from the blood, it is necessary to plot a basic curve, characterizing the colloid in question and serving as a control curve, on the basis of a sufficient number of experimental values. Further, in case of experimental intervention, experimental results should be evaluated by comparison with at least a similar number of controls.

It seems probable that the above considerations are also valid for evaluating the disappearance rate of dyes from the blood in liver function tests.

The disappearance from the blood of the dispersed colloid used is due to the reticuloendothelial cells. We are not acquainted with the mechanism enabling the reticuloendothelial cells to gather the colloid particles from the blood. It is generally concluded that, at least in the first phase, adsorption plays a prominent role, the dynamism of which can be characterised by exponential equations simitar tothe above. Correlations in the living organism are far too complicated, for be ing definitely explained in our days. From the fact that the rate of diminution the colloid in the blood is linear from the 60^{th} minute onward, while the relation is exponential up to the 60^{th} minute (linear of plotted semilogarithmically), it is concluded that the process is not uniform.

Summary

Colloidal lead sulphide colloid traced by ThB was produced. Its disappearance from the blood after intravenous administration and distribution in the reticuloendothelial organs of the rabbit was examined. The conclusions are the following :

1. The greatest affinity to lead salt colloid was observed in the spleen, the bone marrow and the liver. In the second line the storage of the kidney, lungs and adrenals can be considered. The thymus and pituitary do not contain any colloid in the third hour after the injection 27 per cent of the injected 2 mg per kg body weight of colloid is stored in the liver, 1,3 per cent in the kidney, 0,87 in the spleen and 0,57 in the lungs.

2. Analyizing the disappearance from the blood, it was stated that the process can be characterised by two functions, by an exponential one up to the 60th minute and from that time on by a lineal function.

The distribution of colloid in organs and the disappearance from the blood is discussed.

We are much obliged to Professor S. Szalay, director of the Department for Experimental Physics of the University of Debrecen for the preparation of traced radioactive colloid, We are also grateful to Dr. B. Gyires (Mathematical Institute of the University of Debrecen) for the mathematical analysis.

	The activity of the RES organs. Lead sulfid (100 mg/wet tissue)									The storage of the injected 2 mg per kilo body weight lead sulfid in per cent				Remark.
Nr.	Spleen.	Bone marrow	Liver.	Lymph node	Thymus	Kidney	Adrenal	Lung.	Hypophysis.	Liver	Spleen.	Kidney	Lung	
1.	8,12	—	1,65	0,25	0,0	0,64	0,18	∅	∅					♀
2.	9,22	10,7	2,3	∅	∅	0,37	0,48	0,88	∅	43,9	1,5	1,1	1,48	Pregnant
3.	2,4	3,8	2,1	0,25	∅	0,54	0,19	0,24	∅	24,1	0,7	1,5	0,5	♀
4.	2,1	1,7	1,6	∅	∅	0,45	∅	0,65	∅	21,8	0,46	1,5	0,87	♀
5.	2,4	1,4	1,7	∅	∅	0,36	∅	0,21	∅	26,4	0,86	1,2	0,42	♀
6.	2,6	2,6	1,1	∅	∅	0,96	0,13	∅	∅	20,6	0,72	0,3	∅	♀
7.	1,59	0,9	1,7	—	—	0,48	∅	0,15	—	35,7	0,75	1,62	0,35	♀
8.	12,24	2,5	—	—	—	0,47	∅	0,20	∅	16,6	0,5	1,9	0,38	♀
In average:	3,71	3,37	1,7	0,08	∅	0,53	0,12	0,29	∅	27,0	0,78	1,3	0,57	

Table I. The Distribution of traced lead sulphide in the reticuloendothelial organs.

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РАСПРЕДЕЛЕНИЕ МЕЧЕНОЙ СВИНЦОВОЙ СОЛИ В ОРГАНАХ РЕТИКУЛОЭНДОТЕЛИАЛЬНОЙ СИСТЕМЫ

Т. Барка, З. Пошалаки и Л. Кертес

Резюме

Авторы изготовили при помощи тории В меченый PbS коллоид. После внутривенного впрыскивания этого коллоида кроликам, они исследовали его исчезновение из крови и его распределение в органах ретикуло-эндотелиальной системе названных животных. Авторами было установлено следующее:

1. По отношению к коллоидной соли самую большую аффинность показывают селезёнка, костный мозг и печень. Затем следует отложение в почках, легких и надпочечниках. Вилочковая железа и гипофиз не содержат коллоидов. Три часа после подачи 2 мг,кг PbS (коллоидный) из этого количества содержат: печень 1,3%, почка 0,87% и 0,57% легкие.

2. После анализирования исчезновения коллоидных величин из крови было установлено, что этот процесс характеризуется двумя функциями: до 60 минут экспоненциальной, а с этого момента линейной функцией ($y = e^{-0\,039306\,x\,+\,2.634644}$ и $y = -0{,}0118\,x + 2{,}50100$).

Авторы обсуждают сделанные им наблюдения при распределении коллоидов в органах и при их исчезновения из крови.