QUANTITATIVE STUDY OF THE GRANULOPECTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM. I: THE EFFECT OF THE INGREDIENTS PRESENT IN INDIA INK AND OF SUBSTANCES AFFECTING BLOOD CLOTTING *in vivo* ON THE FATE OF CARBON PARTICLES ADMINISTERED INTRAVENOUSLY IN RATS, MICE AND RABBITS.

B. N. HALPERN, B. BENACERRAF AND G. BIOZZI.

From the Laboratory of Experimental Medicine of the Clinique Médicale Propedeutique de l'Hôpital Broussais and Centre National de la Recherche Scientifique, Paris.

Received for publication April 2, 1953.

To investigate the granulopectic activity of the cells which constitute the reticulo-endothelial system various authors have described methods concerned with the injection of vital dyes, or suspensions of particles such as colloidal silver, manganese, India ink, thorium (Cappell, 1929; Wislocki, 1924; Foot, 1920, 1923; Nagao, 1920, Brickner, 1927; Gordon and Katsh, 1949). When particulate matter is injected intravenously, it is the pectic activity of the phagocytes of the reticulo-endothelial system lining the vascular bed which is studied, the suspensions of particles normally used being unable to pass through the vessel walls. In most cases (except in that of thorium) the investigators have studied, by histological methods only, the distribution in the various organs of the particles which had been phagocytosed, without determining either the rate of clearance of the blood or the accumulation in the tissues quantitatively.

We have recently described a method of study of the granulopectic activity of the reticulo-endothelial system in various animal species by injection of a commercial colloidal suspension* of carbon particles of known size (about 200 to 500 Å) (Halpern, Biozzi, Mené and Benacerraf, 1951). Using quantitative methods, the rate of the clearance of carbon from the blood and its accumulation in the various organs were investigated for various doses of carbon. It was found that in rats, when doses of carbon of 16 mg./100 g. or less were injected, the clearance of carbon from the blood was practically complete in an hour and the concentration was an exponential function of the time. Most of the carbon was found in the liver and spleen (90 per cent) while the lungs showed no carbon at all. When doses of carbon above 16 mg./100 g. were injected, the rate of disappearance of carbon from the blood was much more rapid and the curve of clearance could no longer be expressed by a simple mathematical relationship. A considerable amount of carbon was retained in the lungs, kidneys and other organs. Apparently another factor determines the different behaviour of carbon when injected in doses greater than 16 mg./100 g. Doses of ink above 24 mg. of carbon/100 g. constantly caused the death of the animals within a few hours. The same phenomenon was observed in mice, but more irregularly, much less accentuated, and for much higher doses of carbon (above 32 mg./100 g.).

* Commercial India ink, Pelikan, Gunther Wagner, Hanover.

It was suggested (Biozzi, Benacerraf, Mené and Halpern, 1951) that in rats, doses above 16 mg. of carbon/100 g. caused a considerable liberation of thromboplastin which exhausted the reserves of anticoagulants in the blood ; this, added to the considerable surface action of the carbon particles, caused a microcoagulation of fibrin in the blood. Analyses of the amount of fibrinogen in the blood of rats before and after the injection of the various doses of ink showed that the amount of fibrinogen was greatly depleted after the administration of doses of carbon greater than 16 mg./100 g. The same doses of carbon were also accompanied by a considerable decrease in the number of blood platelets.

In mice, in which the paradoxical action of high doses of ink was much more irregular and much less marked, the number of blood platelets was not considerably affected by these high doses of carbon. It is probable that in mice even large doses of ink do not cause a significant coagulation of fibrin.

This mechanism of microcoagulation of fibrin *in vivo* may determine the unexpected action of large doses of ink upon the rate of clearance of carbon from the blood and its distribution in various organs. It is conceivable that the microcoagulation of fibrin *in vivo* causes the formation of larger aggregates of carbon particles and fibrin and modifies in this way the rate of clearance of particles from the blood.

To substantiate this hypothesis we are submitting other evidence based upon the effect on this phenomenon of two groups of substances which either activate or inhibit the coagulation of blood.

All commercial India ink preparations contain mainly carbon particles and and an organic shellac which is a stabilizing agent for the suspension as well as an ingredient which gives lustre to the ink. To investigate whether the blood clotting and toxic effects of the commercial suspensions of carbon generally used by various authors were attributable to the carbon particles or to this shellac, we obtained from Gunther Wagner a special suspension of carbon in fish glue and investigated its properties. We are also reporting data which establish that all untoward reactions observed with high doses of India ink, such as coagulation of fibrin *in vivo*, and toxicity, are attributable to the shellac and not to the carbon particles.

MATERIALS AND METHODS.

Substances which Promote Blood Coagulation.

We have studied the action of thromboplastin (Thrombokinase Hoffman La Roche) and of thrombin (Thrombase Roussel), injected intravenously on the rate of clearance of carbon from the blood and its distribution in the various organs, after standard doses of India ink were administered intravenously. Commercial India ink " Pelikan " which contains 80 mg. carbon/ml. was used in all the experiments. The size of the carbon particles was found to be about 200 to 500 Å by the electron microscope. For animal injection 20 per cent dilutions of ink were made in normal saline containing 1 per cent gelatin, to afford the stabilizing protection of a colloidal agent. Control experiments *in vitro* have shown that within the limits of concentration used, the particles of carbon remain in stable suspension either in serum or in citrated blood. These experiments were carried out on 30 rats weighing 120–140 g. and 15 mice weighing 18–25 g. A dose of 16 mg. carbon/100 g. was given to rats and 24 mg./100 g. to mice ; it has been shown that these doses cause equivalent blood concentrations of carbon in the two animal species. Injections were made into the tail vein.

The rate of clearance of carbon from the blood was investigated. Blood samples were obtained at regular time intervals by puncturing the retro-orbital venous plexus with a capillary glass pipette calibrated to 0·025 ml. and previously washed with heparin (Halpern

and Pacaud, 1951). The blood was lysed in 2 ml. of 0·1 per cent Na_2Co_3 and the amount of carbon in the blood determined electrophotometrically.

The distribution of carbon was studied quantitatively in the liver, spleen, lungs and kidneys for the same doses of ink, by completely digesting the organ in concentrated alcoholic NaOH and finally weighing the amount of carbon recovered (Halpern *et al.*, 1951).

The suspension of commercial thromboplastin (Thrombokinase Hoffman La Roche) was prepared according to the accepted technique for measuring the prothrombin time and 0·2 ml. of this solution was injected intravenously into rats. Commercial thrombin (Thrombase Roussel) was administered intravenously in a dosage of 88 mg./100 g. All the solutions were injected slowly into the tail vein 2 min. before the injection of ink. The animals withstood the injections perfectly well.

Substances which Prevent Blood Coagulation.

We have studied the effect of two anticoagulants, heparin and dicoumarol, on the rate of clearance of carbon from the blood and on its distribution in the organs for various doses of ink. This study was carried out in rats and rabbits, both of which species apparently exhibit the phenomenon of coagulation of fibrin *in vivo* following large doses of carbon (32 mg./100 g. in rats, 16 mg./100 g. in rabbits). The effect of the anticoagulants was not studied in mice because the injection of ink does not cause significant changes in blood clotting consistently.

The experiments were carried out on 45 rats weighing 110–140 g. and on 30 rabbits weighing about 2 kg. Commercial India ink " Pelikan " was used and the dilution made as described above. The doses given to rats were 8, 16 and 32 mg. carbon/100 g. Doses of ink above 16 mg. carbon /100 g. cause a severe thrombocytopenia and a significant decrease in the blood fibrinogen concentration (Biozzi *et al.*, 1951). The rate of clearance of carbon from the blood and its distribution in various organs was investigated as described above. The rats treated with heparin received 25 mg. (Liquemine Roche) into the tail vein 2 min. before the injection of India ink. The study was made on 5 animals for each dose of carbon.

In the rabbit doses of 8 and 16 mg. carbon/100 g. were injected into animals which had previously received 50 mg. heparin intravenously. Blood samples were taken at regular intervals and the amount of carbon in the blood ascertained in the same way as for rats. In the rabbit the injections of carbon were made in the ear vein and the samples of blood taken from the artery in the other ear.

Dicoumarol was given to a group of rats *per os*, 5 mg./100 g. being administered daily for 3 days by gastric cannula. The effect on blood clotting was measured by determining either the blood coagulation time on a slide or the prothrombin time. Only those animals whose blood coagulation time was greater than 8 min. (normal with our technique was 30 sec.) were used for the injection of ink. This corresponded to a prothrombin level below 5 per cent of normal ; 15 animals were used in these experiments, 5 rats for each dose of ink.

Injection of a Suspension of Carbon Particles without Shellac.

A carbon suspension in fish glue was obtained from Gunther Wagner, Hanover (Ink No. C11/1431a). This ink contained approximately 100 mg. carbon/ml. suspended in a solution of fish glue. It was centrifuged at 5000 r.p.m. for 15 min. to remove all particles above 500 Å. The supernatant fluid was then analysed for carbon by weight and dilutions were made in distilled water and gelatin so as to have preparations containing either 16 mg. carbon/ml. in 1 per cent gelatin or 32 mg. carbon/ml. in 2 per cent gelatin. The solutions of gelatin were previously neutralized with ammonia. The preparation containing 16 mg. carbon/ml. was used for doses of 8 mg./100 g. and 16 mg./100 g.; the preparation containing 32 mg. carbon/ml. was used for higher doses. The suspensions were homogenous and stable in serum. They were injected into rats intravenously in the doses described. Care was taken to keep the suspensions at 37° to avoid solidification of the gelatin.

Injections of 8 mg., 16 mg. and 32 mg./100 g. were given to 40 rats. The rate of disappearance of the particles from the blood and the accumulation of carbon in the various organs were studied as previously described. The results (Fig. 9) are presented as the mean for the values obtained, and are compared with those obtained for the same doses of carbon in commercial ink containing shellac.

RESULTS.

Substances which Promote Blood Clotting.

The action of the substances which promote blood coagulation on the rate of clearance of carbon from the blood and its distribution in the various organs was investigated by injecting into rats 16 mg. of carbon/100 g. body weight and comparing the results obtained with those from control animals. It has been established that for this dose of carbon as well as for the smaller doses the curve of clearance of the blood has a simple mathematical shape and the liver and spleen are the only organs which accumulate carbon particles (Halpern *et al.*, 1951).

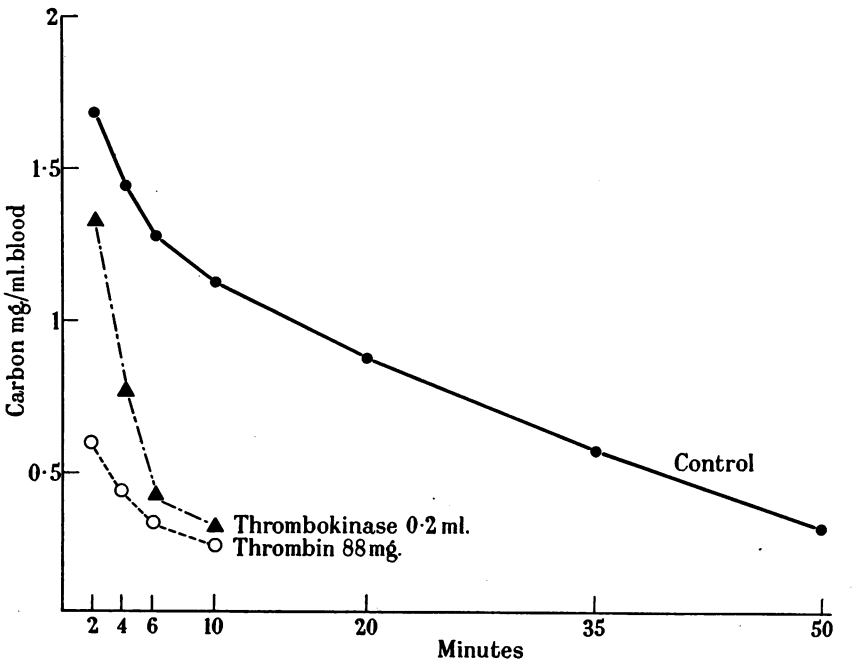


FIG. 1.—Effect of thrombin and thromboplastin (thrombokinase) on the rate of blood clearance of carbon particles in rats following intravenous injection of 16 mg. carbon/100 g. body wt. (commercial ink Pelikan).

We have shown also that this dose of carbon does not change appreciably in rats the amount of fibrinogen in the blood (Biozzi *et al.*, 1951). The results of these experiments are presented as the mean for each group of animals in Fig. 1 and 2.

Following the administration of blood clotting agents and carbon one notes that there is (*a*) much more rapid clearance of carbon from the blood and (*b*) accumulation of more carbon in the lungs than is seen when carbon is injected alone. In mice also thromboplastin causes a rapid disappearance of the carbon particles from the blood (Fig. 3).

The distribution of carbon in the organs of rats following the injection of two different doses of thromboplastin is presented in Fig. 2. In the control animals receiving 16 mg. carbon/100 g., almost all of the injected carbon is found in the liver and spleen. When a large dose of thromboplastin is administered,

considerable quantities of carbon are seen to accumulate in the lungs while the liver and spleen are found to contain less carbon. This behaviour was observed also in mice receiving a corresponding dose of thromboplastin.

The histological studies carried out on the animals treated with thromboplastin were in agreement with the quantitative analysis. In the liver the carbon

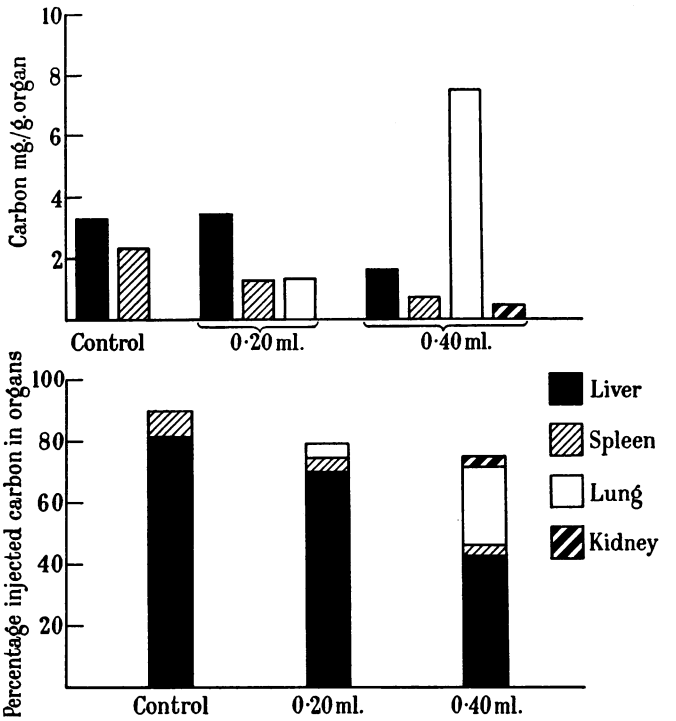


FIG. 2.—Effect of thromboplastin (0·20 ml. and 0·40 ml.) on carbon distribution in various organs of rats following intravenous injection of 16 mg./100 g. (commercial ink Pelikan).

particles were found in the Kupffer cells, more particularly in those located around the portal tracts (Fig. 10). In the control animals this special distribution was not observed (Fig. 11). In the spleen the amount of carbon in the cells appeared much smaller in the animals treated with thromboplastin than in the control animals, which is again in agreement with the results of the analysis by weight.

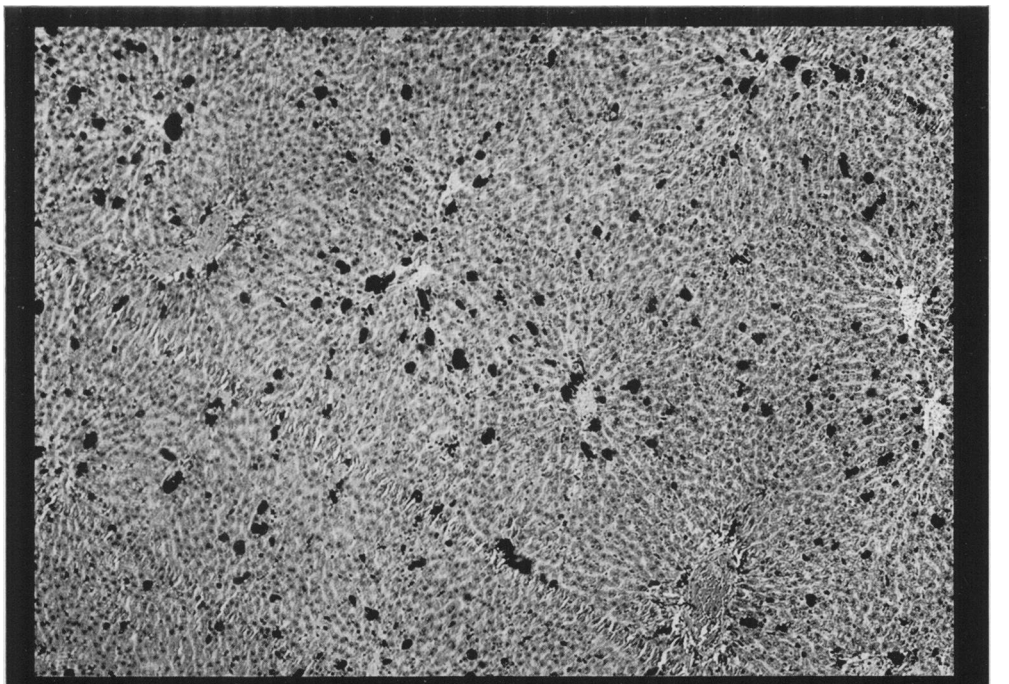
DESCRIPTION OF PLATES.

FIG. 10.—Liver of rat which received intravenously 0·4 ml. thrombokinase followed by 16 mg. carbon/100 g. and was killed 1½ hr. later. Note carbon particles in Kuppfer cells especially those located around portal tracts.

FIG. 11.—Liver of rat given 16 mg. carbon/100 g. intravenously and killed 1½ hr. later. The carbon is evenly distributed in the Kuppfer cells throughout.

FIG. 12.—Lung of rat given 0·4 ml. thrombokinase followed by 16 mg. carbon/100 g., and killed 1½ hr. later. There is a definite quantity of carbon in the capillaries which in places appear lined with carbon.

FIG. 13.—Lung of rat given 0·4 ml. thromboplastin followed by 16 mg. carbon/100 g., and killed 48 hr. later. There is much less carbon than in Fig. 12. Particles of carbon are seen inside intra-alveolar cells; and in a large vessel large cells are filled with India ink.



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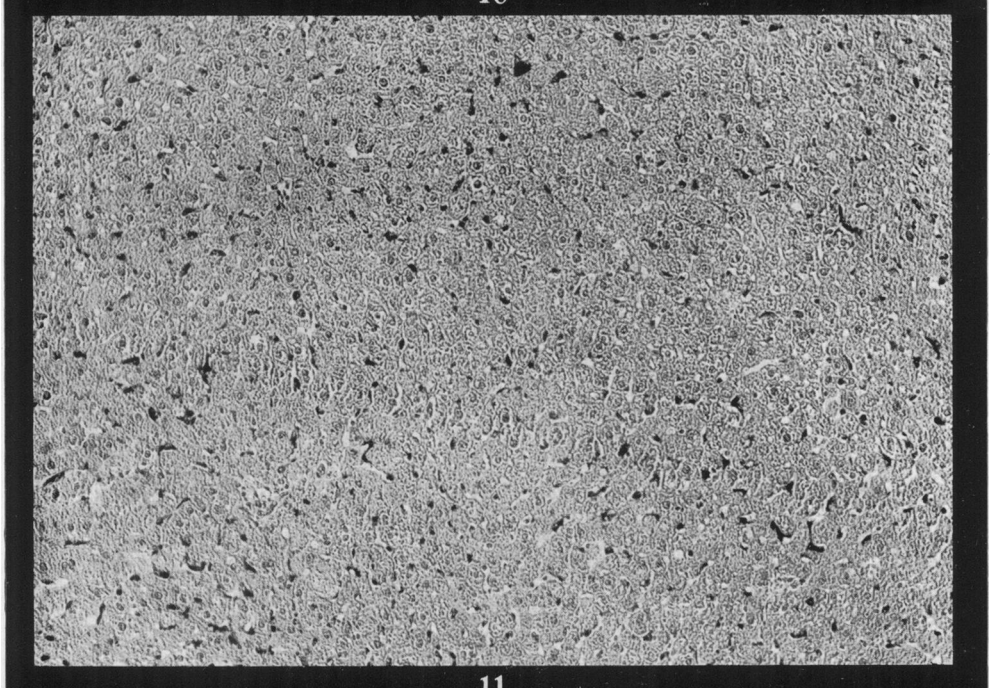

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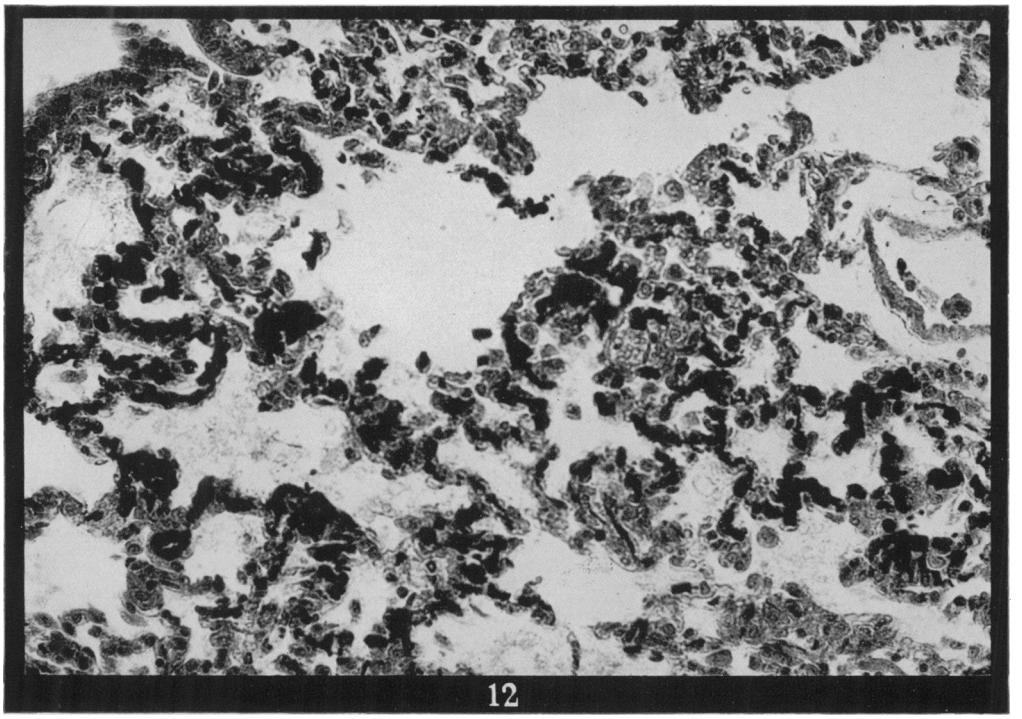

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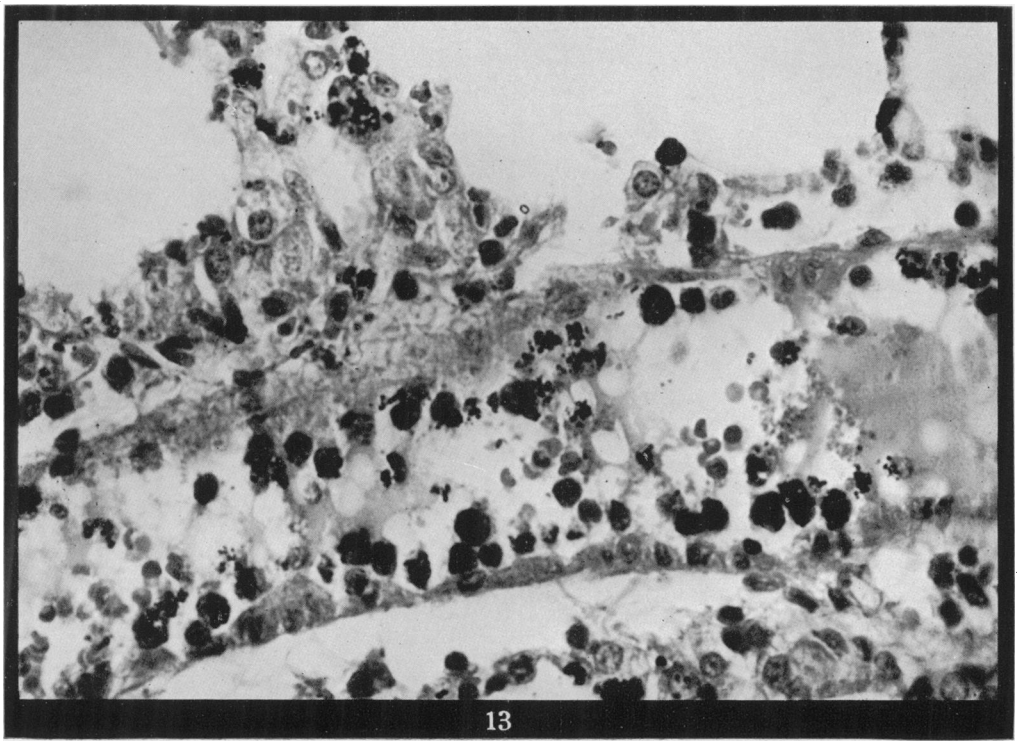

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It should be stressed also that in the animals treated with the coagulant, the capillaries of the lungs were uniformly covered with a layer of carbon (Fig. 12). In the kidneys of these animals, particles of carbon were found in the glomeruli, which seemed to be etched by the carbon.

If the animals are killed 48 hr. later, the histological aspect of the organs is different. A considerable amount of the ink has disappeared from the lungs, but the particles of carbon remaining are inside the intra-alveolar cells. One can observe inside the larger pulmonary vessels some large cells filled with India

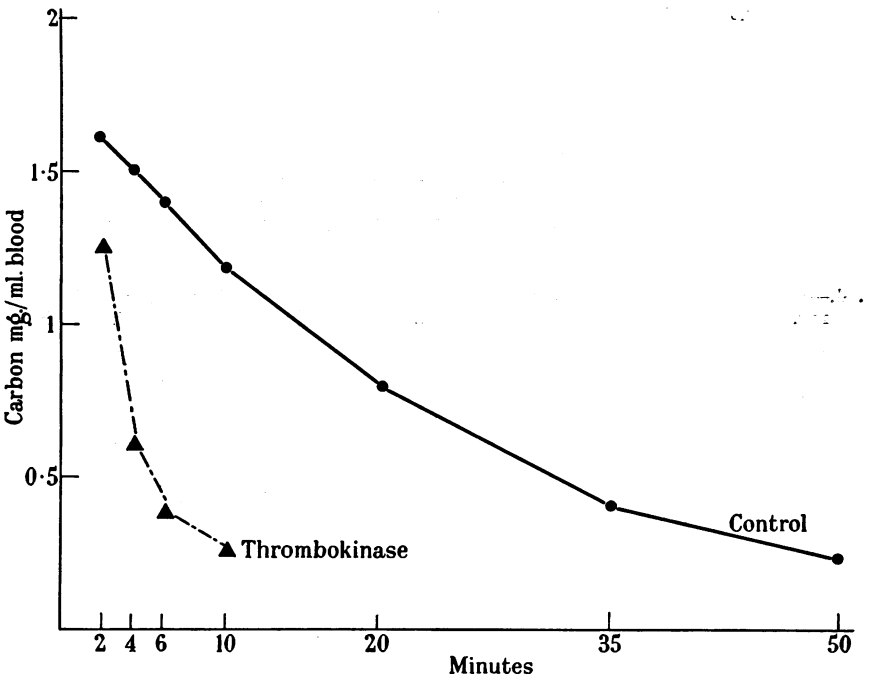

FIG. 3.—Effect of thromboplastin (thrombokinase) on rate of blood clearance of carbon particles in mice following intravenous injection of 24 mg./100 g. (commercial ink Pelikan).

ink (Fig. 13). The liver and spleen, however, showed a greater accumulation of carbon than was found in the animals killed earlier.

In order to verify quantitatively the impression gathered from histological study we analysed the various organs for carbon at regular intervals during the hours following the administration of carbon and thromboplastin (Fig. 4). The values obtained confirm that the carbon particles migrate rapidly from the lungs to the liver and spleen.

Substances which Prevent Blood Coagulation.

Action of heparin in rats.—The rates of clearance of carbon from the blood in heparinized rats are presented in Fig. 5 for various doses of carbon, together with those found in controls. The data represent the mean of the results obtained in 5 rats for each dose.

It can be seen that when 8 mg. carbon/100 g. are injected heparin does not

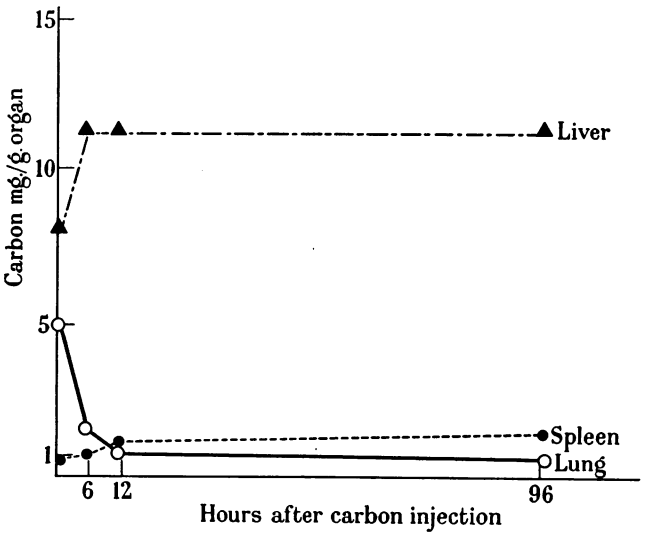

FIG. 4.—Changes in distribution of carbon in rat organs following intravenous injection of 16 mg./100 g. in animals which received an intravenous injection of thromboplastin.

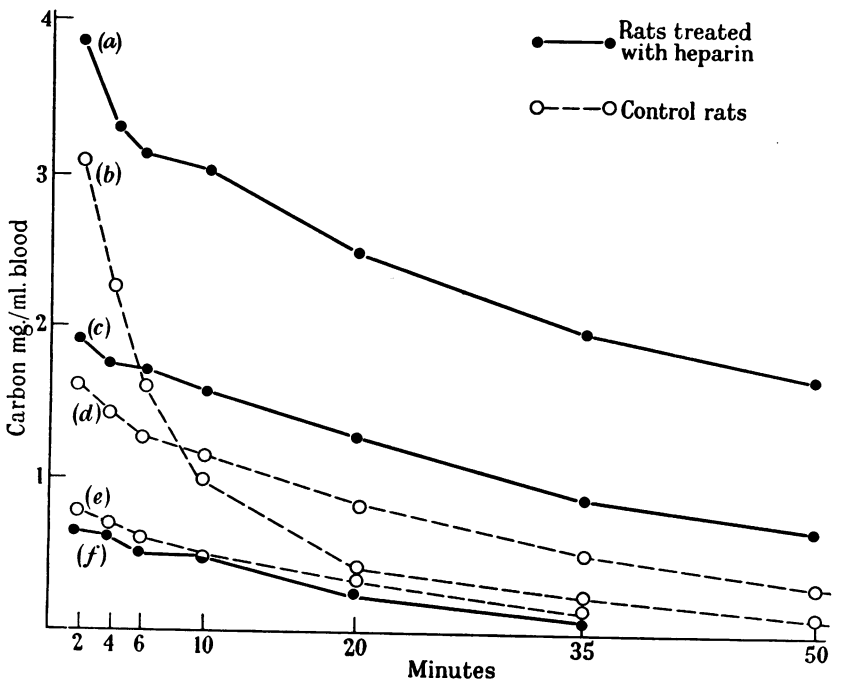

FIG. 5.—Effect of an intravenous injection of 5 mg. heparin on the rate of clearance of carbon from the blood of rats for various doses of carbon (commercial ink Pelikan).

Dosage of carbon : (*a*) & (*b*) 32 mg./100 g.
(*c*) & (*d*) 16 mg./100 g.
(*e*) & (*f*) 8 mg./100 g.

affect the rate of disappearance of carbon particles from the blood. When 16 mg./100 g. are injected into rats previously treated with heparin, the rate of clearance of carbon from the blood is distinctly slowed. This effect is well outside the standard deviation for this dose of carbon (Halpern *et al.*, 1951). The animals treated with heparin which received 32 mg. carbon/100 g. behaved in a way quite different from the control animals. The clearance of the carbon

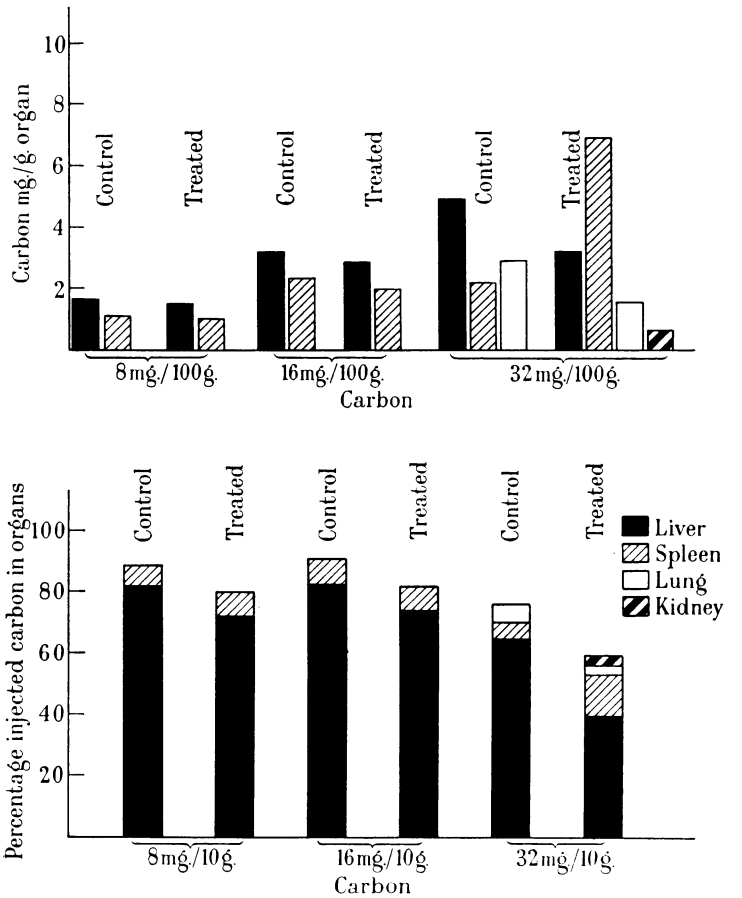

FIG. 6.—Effect of intravenous injection of 5 mg. heparin on distribution of carbon to various organs when different doses of carbon are injected intravenously in rats (commercial ink Pelikan).

particles from the blood was very slow and progressive, and the amount present after 50 min. was still 1·7 mg./ml. as compared with 0·1 mg./ml. in animals not given heparin. This difference is well outside the standard deviation for this dose of carbon.

A study of the distribution of the carbon in the organs is presented (Fig. 6). The analyses were carried out in 6 rats (2 rats per dose). The values are given as the means of the results obtained and compared with those found in control animals receiving the same dose of ink. There is no significant difference in the

distribution of the carbon in the various organs (liver, spleen, lung) between the treated and the control animals when 8 mg. or 16 mg./100 g. are injected. There is a small difference in the total carbon recovered, the amount being less in the treated animals. This can be attributed to the loss of carbon due to the repeated small haemorrhages in the heparinized animals when samples of blood are taken.

There are, however, important differences between the treated and the control rats in the group given 32 mg./100 g. Besides the fact that the total amount of carbon recovered is again lower in animals treated with heparin, there are consider-

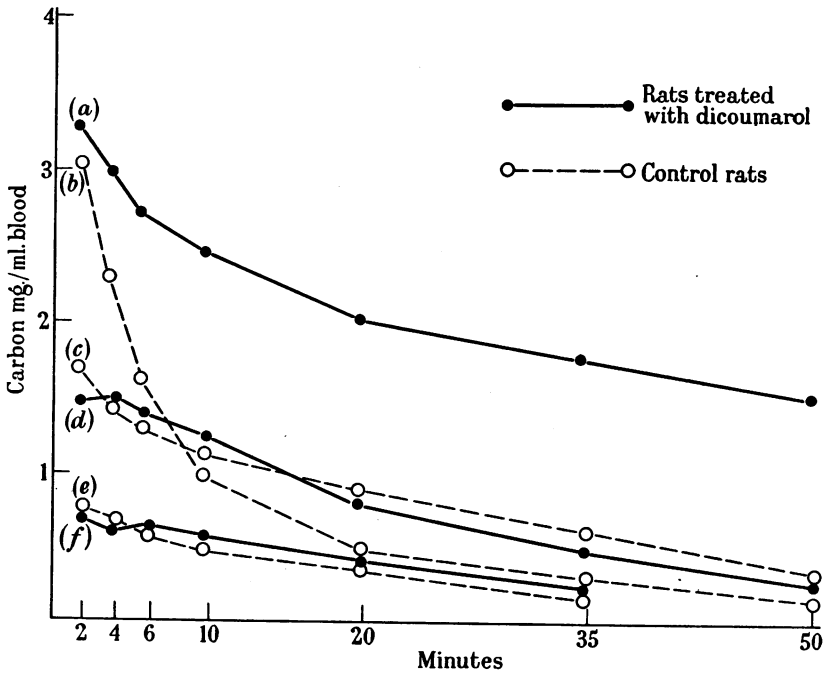

FIG. 7.—Effect of previous treatment with dicoumarol on the rate of blood clearance of carbon particles in rats for various doses of carbon (commercial ink Pelikan).

Dosage of carbon : (*a*) & (*b*) 32 mg./100 g.
(*c*) & (*d*) 16 mg./100 g.
(*e*) & (*f*) 8 mg./100 g.

able changes in the distribution of carbon in the various organs. The lungs of the treated animals contain only about 50 per cent of the amount of carbon found in the lungs of the control rats. On the other hand, the spleen of heparinized animals takes up three times more carbon than that of controls. The liver in animals treated with heparin contains on the average 30 per cent more carbon than in the controls.

Effect of dicoumarol in rats.—The results obtained with dicoumarol resemble those obtained with heparin except that its action is not as effective. The rate of clearance of carbon from the blood is not affected when either 8 mg. or 16 mg. carbon/100 g. are injected. However, when 32 mg./100 g. are injected there is a considerable slowing of the rate of clearance of carbon from the blood (Fig. 7).

In the heparinized animal this effect can be already observed with the dose of 16 mg./100 g. The distribution of carbon in the various organs of the animals treated with dicoumarol is identical with that of the animals treated with heparin for the same doses of ink.

Effect of heparin in rabbits.—When 8 mg. carbon/100 g. are injected, treatment with heparin does not modify the rate of clearance of carbon from the blood in the rabbit (Fig. 8). However, when 16 mg./100 g. are injected the rate of clear-

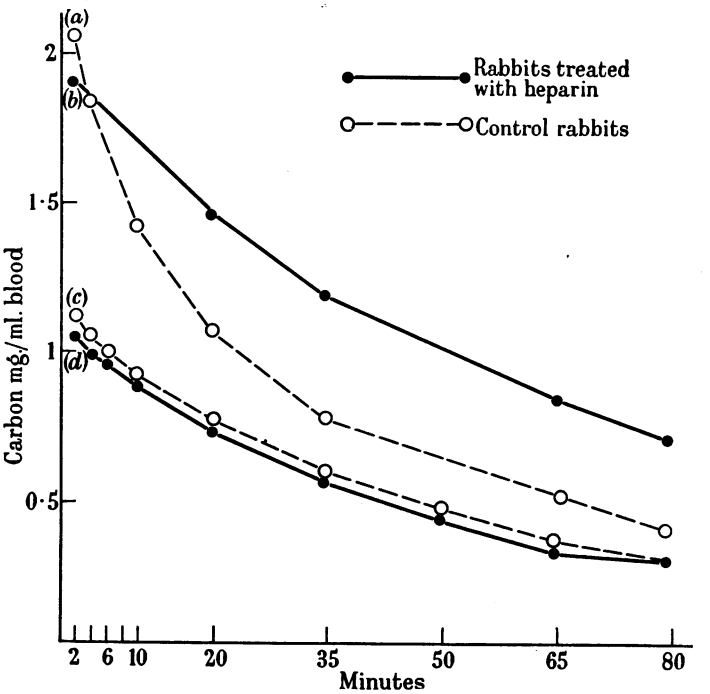

FIG. 8.—Effect of 25 mg. heparin injected intravenously on rate of blood clearance of carbon particles in rabbits when 8 mg. or 16 mg. carbon/100 g. (commercial ink Pelikan) are administered intravenously.

Dosage of carbon: (*a*) & (*b*) 16 mg./100 g.
(*c*) & (*d*) 8 mg./100 g.

ance from the blood is changed appreciably by the previous administration of 50 mg. of heparin. This dose of ink in the rabbit apparently causes a significant microcoagulation of fibrin.

Injection of a Suspension of Carbon Particles without Shellac.

The data presented in Fig. 9 show that when 32 mg. carbon/100 g. of a carbon suspension without shellac is injected, the rate of clearance from the blood differs greatly from that seen when commercial ink containing shellac is administered. When 8 mg./100 g. are injected there is no significant difference between the two curves of clearance. When 16 mg./100 g. are injected the rate of clearance of carbon from the blood in the case of the ink without shellac is somewhat slower

than that seen with the shellac-containing ink, but is identical with the rate of clearance observed in the animals heparinized before receiving the shellac-containing ink (Fig. 5). When 32 mg./100 g. without shellac are injected the rate of clearance is markedly slower than that found in the animals receiving carbon with shellac, and somewhat slower than that of animals treated also with heparin, as heparin is not able to prevent all blood coagulation for this high dose of shellac.

These results show that it is the shellac which is responsible for the phenomenon of blood-clotting *in vivo*, which causes in turn the flocculation of the carbon particles.

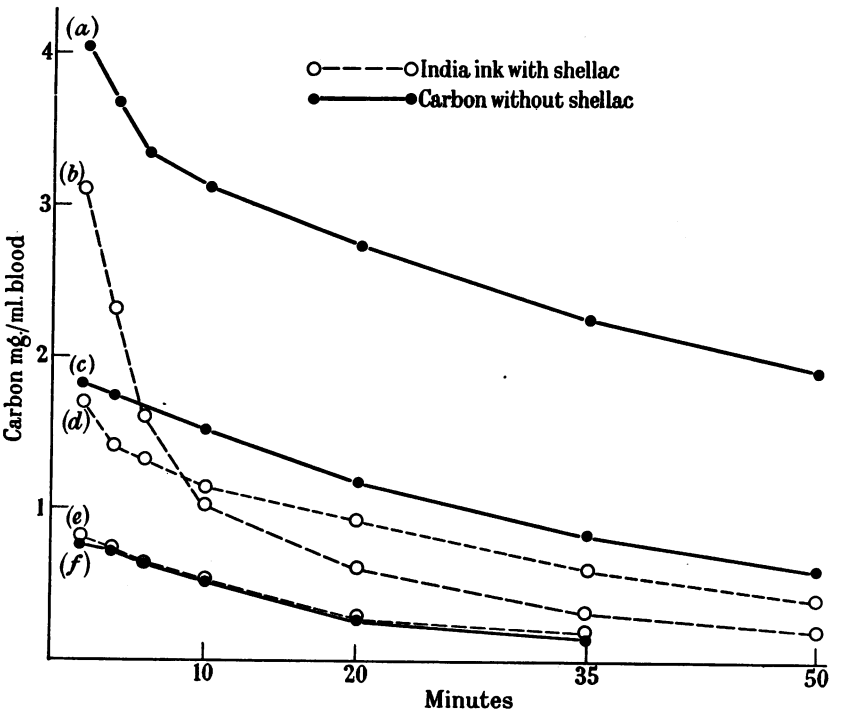

FIG. 9.—Clearance of carbon from the blood in rats following intravenous injection of equivalent doses of carbon as (i) a suspension of carbon without shellac, and (ii) commercial India ink containing shellac.

Dosage of carbon: (*a*) & (*b*) 32 mg./100 g.
(*c*) & (*d*) 16 mg./100 g.
(*e*) & (*f*) 8 mg./100 g.

It should be noted also that the animals given carbon suspension without shellac, even in large doses (up to 48 mg./100 g.) behaved normally and survived indefinitely. This showed that the carbon particles themselves were not toxic.

The amount of carbon recovered from the organs for the various doses of ink showed that there were no differences between animals given ink with shellac and those given the ink without shellac for the doses of 8 and 16 mg. of carbon/100 g. However, when 32 mg. of carbon without shellac are injected only a negligible amount of carbon was found in the lungs and none in the kidneys; almost

all the injected carbon was found in the liver and spleen. When carbon without shellac was administered the carbon was found evenly distributed in the Kupffer cells in the liver and the reticular cells of the spleen for all the doses studied.

A study to determine whether the shellac found in the commercial India ink had coagulative properties showed that these were absent The blood-clotting effect determined *in vivo* by the ink with shellac should therefore be attributed to a release of thromboplastin from the tissues by a mechanism as yet undetermined.

DISCUSSION.

These experiments show that the substances which interfere with the coagulation of blood modify the rate of clearance from the blood of carbon injected intravenously. In order to make this discussion clear it may be recalled that in normal animals (rats, mice, rabbits) there is a critical dose of India ink differing with animal species, below which the rate of clearance of particles from the blood is a regular exponential function of time, and above which the rate of clearance of carbon is considerably accelerated and irregular. With the lower doses of ink containing shellac practically all the carbon is fixed in the reticulo-endothelial cells of the liver and spleen, and with the higher doses of ink a great quantity is retained in the lungs. This radical change is related, as has been shown previously, to an imbalance of the factors which control blood coagulation *in vivo*, and is probably due to a liberation of a great quantity of thromboplastin.

The results presented in this study show that in rats and mice, substances which promote blood clotting render the rate of clearance of carbon from the blood much more rapid and modify the distribution of carbon in the various organs (in the same way as high doses of ink containing shellac in the normal rat).

The substances which promote blood clotting were used in the rat with a dose of 16 mg. of carbon/100 g. which, as we have shown, does not change appreciably the number of blood platelets or the level of plasma fibrinogen. This same effect of the substances promoting blood clotting can be demonstrated using even smaller doses of carbon such as 8 mg./100 g., but in this case the amount of blood-clotting agent to be injected seems to vary inversely with the square of the dose of carbon. When large doses of ink containing shellac are injected or when blood coagulants are injected with small doses of carbon, one can observe the accumulation of carbon in the lungs and kidneys, which normally do not show this phenomenon. The retention of carbon in the pulmonary vessels determined by the coagulation of fibrin is only transient, and slow displacement follows from the lungs to the liver and spleen (Fig. 12). This indicates that the mechanism of the fixation of carbon in the lungs is very different from the true phagocytosis by reticulo-endothelial cells.

Since the substances which promote the coagulation of blood accelerate the clearance of carbon of the blood and modify its distribution in the various organs, one might expect that anticoagulants would effect these reactions in the other direction. We have seen, however that the curve of clearance of carbon for the dose of 8 mg. carbon/100 g. is not changed by anticoagulants, and is identical with that found when the same dose of carbon without shellac is injected. This confirms the fact that in rats and rabbits the dose of 8 mg. carbon/100 g. of commercial India ink does not cause the coagulation of the blood *in vivo*. Moreover it proves also that the phagocytic activity of reticulo-endothelial cells of the liver

and spleen is not affected by a lack of coagulability of the blood, and is not dependent upon the coagulation of fibrin.

When 16 mg. of carbon/100 g. of ink containing shellac are injected into rats treated with heparin, the rate of clearance of carbon from the blood is slower than in control animals although the distribution of carbon in the various organs is not changed. This confirms that this dose of ink causes only a small amount of coagulation of fibrin *in vivo* which slightly modifies the size of the carbon particles, but is not sufficient to cause their retention by the lungs.

Animals treated with dicoumarol with a prothrombin level below 5 per cent of normal do not behave differently from normal animals when a dose of ink of 16 mg. carbon/100 g. is injected. This difference between the effect of heparin and dicoumarol for this dose of carbon can be explained by the difference in the mechanism of these anticoagulants. Heparin is a substance which is believed to inhibit blood coagulation at its various intermediate stages (Eagle, 1937). Dicoumarol is an anticoagulant which acts by blocking the formation of prothrombin. The level of prothrombin remaining in rats treated with dicoumarol may be sufficient to cause the small amount of coagulation of fibrin which is produced in rats by the dose of 16 mg. carbon/100 g.

It must be stressed, however, that both heparin and dicoumarol have a very important effect upon the clearance of carbon from the blood in rats given 32 mg./100 g. in ink containing shellac. In spite of its very powerful anticoagulant action heparin is not able to inhibit the coagulating effect of a high dose of carbon with shellac completely, since a small quantity of carbon is still present in the lungs of these animals. This shows how large a release of thromboplastin is determined by the shellac present in the ink.

The phagocytic power of the spleen appears considerably increased in the heparinized animal or in the animal given carbon without shellac. This greater activity of the spleen can be attributed again to the fact that in these animals the particles of carbon remain in the blood much longer, and not to a greater activity of the splenic cells themselves.

These findings indicate that the mechanism by which carbon particles are taken by the lungs is determined by a coagulation of fibrin *in vivo*. Moreover, it must be emphasized that it is the shellac injected with the carbon particles which is responsible for the liberation of thromboplastin from the tissues by an unknown mechanism.

The following previous work should be reconsidered in the light of these findings. Jancso using colloidal gold reported in 1931 that in mice, treatment with heparin decreased the granulopectic activity of the reticulo-endothelial system. The results of our experiments do not confirm these observations. Recently Sheppard, Gilbert and Hahn (1951) working also with colloidal gold in dogs reported that heparin was unable to influence granulopectic activity.

Timiras and Selye (1949) and Timiras (1953) observed the fixation of carbon particles in the lungs of rats injected with commercial India ink under the effect of " stress," and interpreted it as a true granulopectic activity under the influence of the " alarm reaction." It is quite probable that they have observed a deposition of carbon in the lungs of their animals determined by changes in the equilibrium of the coagulation of blood under the influence of various forms of stress. Moreover the doses of ink used by these investigators alone caused a flocculation of carbon particles *in vivo* and their retention by the lungs.

We suggest that the mechanism demonstrated in this study is responsible for the findings of these authors.

Our experiments also afford a possible explanation for the observations of Sanders, Florey and Wells (1951) and Markham and Florey (1951). These authors reported that when they injected a dilution of commercial India ink into rabbits, they saw the ink disappear very rapidly from the blood. The particles of carbon coalesced to form larger aggregates, and concomitantly they observed an important fixation of carbon by the lungs. It should be mentioned that these authors injected the ink without a protective colloid such as gelatin, and we have observed that coagulation of blood *in vivo* with accumulation of the ink in the lungs will occur with much smaller doses of ink when gelatin is not used to stabilize the suspension.

The rate of blood clearance and distribution of carbon in the organs are not affected either by anticoagulants or by the presence of shellac when a dosage of 8 mg./100 g. is employed. This indicates that when using commercial ink to study the phagocytic activity of the liver and spleen this dosage must not be exceeded if errors caused by coagulation of fibrin *in vivo* are to be avoided. If higher dosage is required it is preferable to use a suspension of carbon without shellac such as the one we have described in this study.

SUMMARY.

Thrombin or thromboplastin, when administered intravenously, increase the rate of blood clearance of carbon particles and modify the distribution of carbon in the organs.

The action of substances promoting blood coagulation is therefore very similar to that of high doses of commercial India ink (carbon with shellac) alone. This confirms the hypothesis that high doses of these inks cause a microcoagulation of fibrin *in vivo*.

The action of anticoagulant differs according to the dose of India ink injected. Small doses of carbon modify neither the rate of clearance of carbon particles of the blood nor their distribution in the various organs. When high doses of ink are injected a considerable decrease in the rate of clearance of carbon of the blood is observed. The amount of carbon in the lungs of animals treated with the anticoagulants is smaller and that in the spleen greater than in controls.

The coagulation of fibrin *in vivo* caused by higher doses of commercial India ink as well as its toxicity are attributable to its shellac content.

In a study of the activity of the reticulo-endothelial system only specially prepared suspensions of carbon without shellac should be used. If commercial India ink containing shellac is injected, care should be taken to administer doses which do not cause a release of thromboplastin *in vivo*.

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