CXLVI. STUDIES ON BAYER 205 (GERMANIN) AND ANTRYPOL[1]

IV. THE RETENTION OF THE DRUG IN THE ANIMAL BODY

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IN previous papers [Dangerfield *et al.* 1938; Boursnell *et al.* 1939] a method for the determination of Bayer 205 was described, and observations were made on the persistence of this drug in the blood stream after injection into animals. The long retention of the drug in the body is of special interest in view of the prolonged protective effect against trypanosomiasis produced by the injection of Bayer 205, and it was considered desirable that further investigations should be carried out to determine the cause of this retention. The prophylactic value of this drug, for man and other animals, has been the subject of a large number of reports [for the literature, cf. Findlay, 1930, p. 271; 1939, p. 205], and most authorities agree that the injection of 1 or 2 g. will protect man against trypanosome infection for several weeks, and in some cases for several months. This long protective action has usually been explained by the assumption that there is retention of the drug by the body, and support for this view has been given in earlier papers of this series.

The molecule of Bayer 205 (mol. wt. 1428) is relatively large, but not sufficiently large to suggest that the drug will not be excreted by the kidney; indeed there is evidence that appreciable quantities are excreted in the urine during the first few days after injection. Some other explanation must therefore be sought to account for retention in the body. The experiments described in this paper have been carried out to determine (*a*) whether there is any appreciable storage of the drug in the liver, kidney and other organs and tissues, and (*b*) whether persistence in the blood stream is due to combination of the drug with plasma proteins.

EXPERIMENTAL

Bayer 205 determinations

On plasma, serum and protein precipitates. These determinations were carried out as described in previous papers [1938; 1939]. Distilled water was added to bring the volume to about 3·0 ml. before the addition of 3·0 ml. of conc. HCl. In most of these determinations "Analar" HCl has been used, after dilution to 10·4*N*, the strength of the conc. HCl hitherto employed; there is no suggestion, however, that the ordinary pure HCl is not suitable for this determination.

[1] This communication represents a third report to the Medical Research Council at whose request this work was undertaken.

All the measurements were made with the Lovibond tintometer discs previously described. Compensation for the small blank value for normal plasma or serum (equivalent to about 1·0 mg. Bayer 205/100 ml.) was effected by a control tube containing a corresponding amount of hydrolysed, diazotized and coupled normal plasma, serum or plasma-protein. The hydrolyses or diazotizations were usually carried out in duplicate and these almost invariably showed satisfactory agreement. The results recorded in the tables are average values for two or more determinations, and have been corrected, unless otherwise stated, for the blank values for normal serum, plasma etc.

On tissues. As soon as each rabbit was killed, various organs were removed,[1] dried on filter paper and weighed. Weighed amounts of the tissues (usually 1·5–2·5 g. except where the amount available was small) were quickly transferred to stoppered pyrex tubes graduated at 10 ml. Water was added to give a volume of about 3 ml., and 3 ml. of 10·4*N* HCl were added at once to prevent bacterial or enzymic changes in the tissue. After hydrolysis many of these solutions had a very dark colour which was not completely removed by treatment with kaolin. To compensate for this colour, a control mixture was prepared for each hydrolysate, containing all the reagents used for the colorimetric determination except sodium nitrite, and when this control solution was placed in the appropriate part of the comparator, satisfactory matching of the colours could be made. For several reasons it was not possible to compensate for the blank values of normal tissues by the simple procedure used with plasma or serum, and it was necessary to carry out determinations on the tissues of normal rabbits kept under the same conditions as the injected animals. The results recorded in Tables I and II represent, therefore, values for Bayer 205 plus other "amine-precursors", but the values for normal tissues are sufficiently constant (cf. Table I) to justify the subtraction of "blanks" for each tissue, to give values which represent the Bayer 205 content of the tissues of the injected rabbits (Fig. 1). The source of these amines present in hydrolysed normal tissues has not been investigated, but it is probable that they originate mainly from protein, as they do in the case of normal plasma. The recovery of Bayer 205 added to various normal tissues is not as good as the recovery from plasma, but it is sufficiently satisfactory for the purposes of this investigation. Amounts of Bayer 205 equal to those found in the tissues of the injected rabbits are recovered to the extent of 80–90 % with most tissues (kidney, heart, brain etc.), but the recovery with liver tissue is only 55–65 %.

In these experiments on "storage" in the tissues, and in many others, Antrypol has been used, and in this work the two terms (Antrypol and Bayer 205) are used synonymously.

The Antrypol content of various organs and tissues following the injection of the drug into rabbits

Three groups of rabbits received intravenous injections of Antrypol solutions at intervals; at each injection 0·03 g. Antrypol/kg. was given. The injections were made at intervals of 3 weeks, and two groups were killed 3 weeks after they had received the last injection. The third group, that receiving three injections, was left for 10 weeks after the last injection, since it was thought advisable to avoid a high plasma-Bayer 205 level which would cause high values

[1] The authors would like to take this opportunity of thanking Dr J. L. D'Silva of the Department of Physiology for his generous assistance with these operations.

to be obtained for tissues rich in blood. The weights of the rabbits ranged from 1·7 to 3·1 kg. The details of the injections can be tabulated as follows:

	No. of rabbits	No. of injections	Intervals between injections days	Interval between last injection and death days
Group A	3	1	—	21
Group B	3	2	21	21
Group C	4	3	21 and 21	70

Two control rabbits for each group were kept under similar conditions with regard to diet etc., and were subsequently treated in the same way as the injected animals. The results of this experiment are given in Tables I and II and in Fig. 1. With the injected rabbits, as with the controls, there is good agreement between two samples of the same organ, although these samples were often taken from different parts of the organ concerned. If the values for the kidney are excluded, the average difference between duplicates of this type is less than 0·04 mg./g. of tissue.

The "true" Antrypol values for the organs of the injected rabbits are plotted in Fig. 1. These figures represent the differences between the averages for each organ of each group of injected rabbits and the corresponding average "blank" values for the control animals (last column, Table I). The results obtained show that there is a small amount of the drug in each of the organs examined, with the possible exception of the brain. Part of the Antrypol present in each tissue can certainly be attributed to the blood and tissue fluid of that organ, but there is usually sufficient to indicate a slight retention of the drug in the tissues in addition to that present in the blood. It is suggested that this retention is due to combination with tissue proteins, but with two exceptions the amount of drug retained in any one organ is very small. The values for the kidney, however, are considerably higher than those for any other organ or that for plasma, and in addition there is distinct variation between the values for different rabbits of the same group (cf. Table II). The spleen also gives higher results than those for

Table I. *Amines produced by acid hydrolysis of tissues of normal rabbits*

Calculated as mg. Bayer 205/g. tissue or/ml. of plasma

	No. 170	No. 177	No. 185	No. 188	No. 297	No. 299	Average
Kidney	0·070	0·033	0·048	0·042	0·039	0·041	0·043
	0·031	0·029	0·052	0·043	0·039	0·044	
Liver	0·039	0·042	0·050	0·035	0·032	0·038	0·040
	0·041	0·046	0·050	0·039	0·034	0·036	
Heart	0·033	0·033	0·055	0·037	0·047	0·048	0·040
	0·034	0·033	0·039	0·038	0·047	0·040	
Muscle*	0·022	0·030	0·034	0·024	0·035	0·016	0·026
	—	—	0·023	0·027	0·032	0·015	
Lung	0·048	0·032	0·035	0·034	0·036	0·042	0·039
	0·050	0·035	0·041	0·036	—	0·039	
Brain	0·036	0·022	0·029	0·028	0·028	0·025	0·028
	0·028	0·034	0·026	0·027	—	0·026	
Adrenals	0·033	0·029	0·012	0·034	0·016	0·015	0·023
Spleen	0·054	0·063	0·061	0·069	0·042	0·070	0·060
Pancreas	0·031	0·016	0·024	0·029	0·030	0·032	0·027
Plasma	0·008	0·008	0·008	0·008	0·008	0·007	0·008

* From the abdominal wall.

Table II. *Tissues of rabbits injected with Antrypol (Antrypol plus other amine-precursors in terms of mg. Bayer 205/g. tissue or/ml. of plasma)*

	Group A (1 injection) Rabbit no.			Group B (2 injections) Rabbit no.			Group C (3 injections) Rabbit no.			
	244	282	283	229	237	241	285	289	301	317
Kidney	0·239	0·139	0·111	0·111	0·290	0·124	0·104	0·119	0·099	0·116
	0·210	—	0·114	0·122	0·310	0·103	0·105	0·116	0·102	0·123
Liver	0·059	0·069	0·049	0·064	0·067	0·060	0·038	0·055	0·044	0·057
	0·047	0·068	0·047	0·056	0·063	0·055	0·041	0·056	0·041	0·058
Heart	0·062	0·066	0·058	0·061	0·063	0·062	0·054	0·062	0·047	0·057
	0·059	0·068	0·053	0·073	0·064	0·062	0·049	0·064	0·049	0·049
Muscle	0·035	0·052	0·043	0·061	0·058	0·053	0·018	0·030	0·014	0·017
	—	—	0·040	—	0·046	0·047	0·026	—	0·017	0·024
Lung	0·060	0·056	0·053	0·071	0·068	0·072	0·040	0·051	0·050	0·055
	0·065	0·056	0·050	0·071	0·061	0·068	0·044	0·049	0·050	0·055
Brain	0·029	0·029	0·026	0·034	0·027	0·027	0·026	0·031	0·027	0·024
	0·029	0·032	0·029	0·032	0·032	0·029	0·026	0·027	0·028	0·025
Adrenals	0·035	0·047	0·047	0·072	0·063	0·054	0·026	0·030	0·029	0·073
Spleen	0·129	0·107	0·089	0·155	0·091	0·114	0·073	0·115	0·123	0·167
Pancreas	0·034	0·043	0·034	0·059	0·045	0·046	0·034	0·041	0·034	0·033
Plasma	0·040	0·037	0·031	0·056	0·048	0·049	0·020	0·020	0·019	0·022

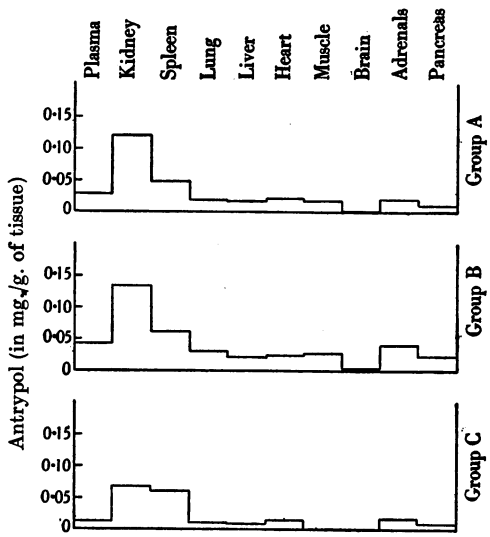

Fig. 1. The Antrypol content of the tissues of rabbits which had received injections of the drug.

plasma, but not as high as those for the kidney. In the case of two of the rabbits, determinations were also carried out with bone marrow and a portion of small intestine; values similar to those for heart, liver, muscle etc. were obtained.

The combination of Bayer 205 with plasma proteins

For this investigation several methods have been used. In some experiments Bayer 205 has been added to serum (rabbit and horse), and in others blood was taken from rabbits which had previously received injections of the drug. The

plasma and serum proteins were then separated by various methods and the amount of Bayer 205 in each fraction was determined. In many of the experiments no attempt was made to find the relative combining capacities of the different proteins for Bayer 205, the main object being to determine whether the drug can combine with plasma proteins. In view of the anticoagulant action of this compound, however, particular attention has been paid to the fibrinogen fraction.

(*a*) *Bayer 205 added to serum.* Normal horse serum was treated with 0·04 vol. of a strong Bayer 205 solution and kept at room temperature for 1 hr.; the globulins were then separated from portions of this mixture by CO_2. The results recorded in Table III indicate that a considerable amount of the added drug attaches itself to the globulins. Washing with water saturated with CO_2, or reprecipitation with CO_2, removes part only of the "attached" drug, and the observed loss is largely due in the first case to solution of the globulin-drug complex, and in the second to the difficulty in effecting complete reprecipitation of the globulin.

Table III. *Separation of the globulins from serum to which Bayer 205 has been added*

	mg. Bayer 205/100 ml. serum*			
Whole serum (horse)	93	1·7	48	97
Globulin fraction (CO_2)	53	1·2	17	26
Globulin fraction (washed)	46	1·2	17	22
Globulin fraction (reprecipitated)	35	1·2	13	21

Experimental details. A mixture of 1 vol. of serum and 9 vol. of distilled water was cooled in ice-water, and the globulins were precipitated by passing in CO_2 to give maximum precipitation. The mixture was kept in ice-water for 20 min. and centrifuged. The deposit was dissolved in 1 vol. of 0·9 % NaCl with the aid of sufficient dilute NaOH to give *p*H 7·5.

Some of the globulin precipitates were washed with CO_2-saturated distilled water, and in other cases reprecipitation was effected by dilution and treatment with CO_2.

* In these experiments and those recorded in Tables IV–VI, blank determinations have been made on the corresponding fractions of normal plasma or serum, and compensation has been made for the small amount of amine-precursors present in some of these controls.

In other experiments the drug was added to horse (and rabbit) serum and the proteins precipitated by alcohol. Precipitation was effected by adding 3 vol. of absolute alcohol, and the precipitates were washed with absolute alcohol (4 vol.) and finally with ether (4 vol.). With greatly varying amounts of Bayer 205 (1–90 mg./100 ml.) the protein precipitates contained almost all (93–95 %) of the added drug.

(*b*) *The plasma of rabbits injected with Bayer 205.* Blood was taken from rabbits which had previously received one or more intravenous injections of Bayer 205, the conditions of the experiment being varied as much as possible to obtain a wide range of plasma-Bayer 205 values.

Precipitation of the plasma proteins by alcohol gave preparations which contained 95–100 % of the plasma-Bayer 205. This combined or adsorbed Bayer 205 was not removed to any marked extent by washing with alcohol, and less than one-third was removed when the precipitates were washed with aqueous alcohol (Table IV).

In other experiments various protein fractions (fibrinogen, fibrinogen plus globulin, and globulin) have been separated from Bayer 205-containing blood from injected rabbits. The results recorded in Table V show that an appreciable

Table IV. *Bayer 205 content of alcohol-precipitated proteins of the plasma of rabbits which had received injections of the drug*

	mg. Bayer 205/100 ml. plasma								
Plasma	2·9	1·7	10·3	12·8	7·75	6·8	5·0	4·75	3·75
Precipitated proteins:									
(*a*)	2·85	1·55	9·7	12·6	7·8	6·6	—	—	—
(*b*)	—	—	9·1	11·9	—	6·5	4·8	4·55	3·65
(*c*)	—	—	—	—	—	—	3·45	3·25	2·85

Experimental details. 2 ml. oxalated plasma were treated with 6 ml. of absolute alcohol, mixed well and centrifuged. Some of these precipitates were treated as follows:

(*a*) Dried *in vacuo* over $CaCl_2$.

(*b*) Washed with 8 ml. of absolute alcohol, then with 8 ml. of ether and dried at room temperature.

(*c*) Washed successively with 8 ml. of "75% alcohol" (1 vol. of water plus 3 vol. of alcohol), 8 ml. of absolute alcohol and 8 ml. of ether; dried at room temperature.

part, but not the whole, of the drug present in the plasma is attached to the globulin. Of the other plasma proteins the most interesting in this connexion is fibrinogen, since it has been suggested that the anticoagulant action of Bayer 205 is due to the combination of the drug with fibrinogen. It has previously been noted, however, that plasma and serum obtained from the same blood contain the same amount of Bayer 205 [Boursnell *et al.* 1939], and further determinations covering a much wider range of Bayer 205 levels, and with considerable variation in the number of injections have fully confirmed this observation (cf. Table VI). From these results and from determinations on fibrinogen (fibrin) precipitated by the addition of $CaCl_2$ to the diluted plasma (Table V), it is concluded that under the conditions of these experiments no significant part of the plasma-Bayer 205 is closely attached to the fibrinogen. The small amount present in the precipitated fibrin may possibly be associated with some component of fibrin other than fibrinogen.

Table V. *Bayer 205 content of separated proteins of the plasmas of injected rabbits*

	mg. Bayer 205/100 ml.							
Serum	—	—	5·0	3·9	—	—	—	—
Plasma	2·9	1·7	—	—	18·2	22·2	15·2	12·2
Fibrinogen (fibrin)	0·3	0·3	—	—	1·0	0·9	0·8	0·3
Fibrinogen *plus* globulin	1·0	0·75	—	—	2·85	2·6	3·0	1·7
Globulin	—	—	1·5	0·95	—	—	—	—

Experimental details. Fibrinogen (fibrin) was separated by the addition of $CaCl_2$ to plasma diluted with 0·85% NaCl, and fibrinogen plus globulin by the addition to plasma of 19 vol. of 27·79% $(NH_4)_2SO_4$ solution [for full details, cf. Harrison, 1937]. Serum globulin was separated by the addition of 1 vol. of water and 2 vol. of saturated $(NH_4)_2SO_4$ solution, and the precipitate was washed with about 3 vol. of half-saturated $(NH_4)_2SO_4$ solution.

Table VI. *Comparison of plasma and serum (of injected rabbits)*

	mg. Bayer 205/100 ml.							
Plasma (oxalated)	7·0	9·3	5·4	4·5	28·4	15·0	21·1	15·8
Plasma (heparinized)	—	9·3	—	4·6	—	15·0	20·3	—
Serum	7·0	—	5·2	—	28·6	—	—	16·0

Experimental details. Samples of 2·5, 5·0 and 2·5 ml. of blood were taken from each rabbit. The first and third samples were oxalated and mixed; the second sample was collected in a heparinized tube (containing 0·05 ml. of 1% heparin solution) or in a clean tube (for serum separation).

Discussion

Little information is available as to the fate of Bayer 205 in the animal body, and although it is known that a considerable amount of the injected drug remains in the body, the cause of this retention is still rather obscure. Sei [1923] has shown that a trypanocidal action is exerted by extracts of certain organs of animals which have received large doses of Bayer 205, and chemical tests have been used to detect the drug in the tissues of the injected animals [Zeiss & Utkina-Ljubowzewa, 1930; Demidowa, 1931]. These chemical tests were made, however, by a method which does not appear to be satisfactory for the determination of Bayer 205 [cf. criticism by Lang, 1931; Dangerfield *et al.* 1938], and, furthermore, in practically all these earlier investigations the animals were injected with large doses of the drug.

The method of determination of Bayer 205 evolved in previous papers [Dangerfield *et al.* 1938; Boursnell *et al.* 1939] can be utilized for determinations on tissues. The recovery of added Bayer 205 is not as satisfactory as it is in the case of plasma, but is sufficiently high (80–90 % with most tissues) for practical purposes. The method will detect very small amounts of Bayer 205 and it has been possible, therefore, to limit the amount of injected drug to a normal prophylactic dose. At each injection the rabbits used in this investigation received 0·03 g. of drug/kg., equivalent to a dose of about 2 g. for an average man. One disadvantage of this or any similar chemical determinations is that it does not differentiate between Bayer 205 and possible modifications of the drug, but there is no evidence as yet that degradation of Bayer 205 occurs in the animal body. It is hoped in a later publication to deal with this question of the possible "metabolism" of this drug.

The determinations of the Antrypol (or Bayer 205) content of the tissues of rabbits which had previously received one, two or three injections of the drug show that there is no preferential storage of the drug in the liver or any other organ examined, with the possible exception of the kidney. The amount of drug in the liver, heart, muscle, lung, adrenals and pancreas is very small, and the average value for each of these tissues is usually 0·5/0·9 times that present in an equal weight of blood (cf. Fig. 1). The drug present in the blood and tissue fluid can undoubtedly account for an appreciable part, but not all, of the total drug present in each of these organs. It seems probable, therefore, that a very small amount of the drug is held in each tissue, partly in combination with the proteins of blood and tissue fluid, and possibly associated with the tissue proteins; it is significant in this respect that brain tissue which contains very little protein contains little or no Antrypol. The Antrypol content of the liver and other similar tissues is, however, very small, and represents only a fraction of the injected drug. Three weeks after the injection of 30 mg. of Antrypol/kg. of body weight, the liver contained about 0·8 mg. and the heart 0·1 mg. of the drug (group A). The corresponding figures, 3 weeks after a second injection of the same amount (group B), are 1·2 and 0·1 mg. respectively, and for the third group, where three injections were given and the rabbits were killed 10 weeks after the third injection, the values were 0·6 and 0·07 mg. respectively. The corresponding values for the whole of the blood, assuming a blood volume of approximately one-twelfth of the body weight, are 5·5, 8·3 and 2·4 mg. for groups A, B and C respectively. As was mentioned above, these experiments were carried out to determine whether there is appreciable storage of Bayer 205 in any particular organ, and it was not intended that they should serve as balance experiments to determine exactly how much of the drug is retained in various parts of the body.

Experiments of an entirely different nature are being carried out with the latter object in view, but from the observations made in the present paper it can be concluded (*a*) that the drug is fairly widely distributed throughout the body, and (*b*) that for several weeks after the injection of the drug a significant part of the injected material is still present in the animal. It is hoped that subsequent investigations on the urinary excretion of the drug by man, and other animals, will indicate how much of the injected drug remains in the body after a period of several weeks.

Of all the organs examined, the kidney occupies a special position. The values obtained suggest that significant amounts of the injected drug often accumulate in this organ, particularly in certain animals; thus one rabbit (no. 244), which received 82 mg. of Antrypol, had 2·2 mg. of the drug in its kidneys 3 weeks later, and another rabbit (no. 237) which received two injections each of 90 mg., had 3·8 mg. of the drug in its kidneys 3 weeks after the second injection. The presence of appreciable amounts of the drug in the kidney is of special interest in view of the fairly frequent occurrence of albuminuria in patients treated with the drug. There is also evidence that the injection of Bayer 205 into healthy mice causes extensive degeneration in the epithelium of the convoluted and other secreting tubules of the cortex of the kidneys [Duncan & Manson-Bahr, 1923–4].

The amount of drug in the spleens of the injected animals is not as high as that in the kidneys, but it is significantly higher than the values for the other organs examined. Part of this difference can be attributed to the high blood content of the spleen, but it is possible that some additional factor is concerned. There is much recent work which suggests that the spleen, and the reticulo-endothelial system in general, play an important role in the chemotherapeutic action of certain trypanocidal drugs, and it is claimed that with rats and mice, splenectomy destroys the sterilizing action of various trypanocidal drugs [for the literature see Findlay, 1939, p. 231]. According to Kritschewski [1928] combination of the drug with agar gives effective results in these splenectomized animals, presumably by the formation of a depot from which slow absorption occurs. There is also evidence that Bayer 205 may exert its action by an opsonin-like effect, producing a change in the trypanosomes which renders them more readily phagocyted [Reiner & Köveskuty, 1927; Jancsó & Jancsó, 1934; 1935; Hawking, 1939]. According to this view the reticulo-endothelial system would play an active part in the trypanocidal effect but it would not necessarily suggest that Bayer 205 combines with the reticulo-endothelial system. Although they offer no evidence as to the role of the spleen in the chemotherapeutic action of Bayer 205, the experiments described in the present paper indicate that there is no extensive storage of the drug in the spleen.

From the above results it is concluded that the long retention of Bayer 205 in the animal body following intravenous injection of the drug is not due to storage in any particular organ, but to a more general retention in the blood and tissues. One possible explanation for this retention is that the drug combines with the plasma- and tissue-proteins, and for many reasons this possibility has been under consideration throughout this work. It is of interest to note that in some of the earliest investigations on Bayer 205 it was suggested that the drug is bound up with the serum proteins, and a considerable amount of indirect evidence in support of this view has been obtained by many workers in investigations on the trypanocidal, anti-complementary and anti-coagulant action of the drug. In certain concentrations, Bayer 205 prevents the heat-coagulation of blood proteins [Collier, 1925] and it also protects these, and other proteins, against

precipitation by tannin, mercuric chloride and certain other reagents [Jirovec & Kocian, 1930; Kocian, 1936]. Klopstock [1932] has investigated the effect of Bayer 205 and of heparin on immune reactions and concludes that both substances shift the isoelectric point of certain of the immune substances to the acid side, and that in higher concentrations they react with specific groups in the protein molecule. There is also evidence that Bayer 205 specifically inactivates certain enzymes. Quastel [1931], for example, found that it is toxic to fumarase but not to urease, and he suggested that there is some structure in common between the fumarase enzyme, cotton fibre and the trypanosome which makes for specific combination or adsorption with certain naphthylaminedisulphonic acid derivatives. Fürth *et al.* [1932] also report that Bayer 205 is not a general enzyme poison but that it accelerates post-mortem production of acid in muscle and liver, and the bacterial production of lactic acid in milk.

In the experiments reported in this paper, evidence is produced that Bayer 205 is closely associated with plasma proteins. It has been found that the proteins precipitated from normal plasma or serum after the addition of the drug, or from the plasma or serum of injected rabbits, contain appreciable amounts of Bayer 205. For obvious reasons the methods of precipitation were varied as widely as possible, and it is believed that there can be no possibility that in every case the drug was carried down by "simple" adsorption. If, however, it is suggested that adsorption accounts in every case for the drug present in the precipitates it would still indicate a fairly strong chemical affinity between the protein and the drug. The proteins precipitated by the addition of 3 vol. of alcohol contain practically all the Bayer 205 present in plasma or serum, and the drug is only partially removed when the precipitate is washed with "75%" alcohol (in which Bayer 205 is quite soluble). The globulin precipitates obtained with ammonium sulphate also retained a high proportion of the drug when they were washed with ammonium sulphate solutions, and solution and reprecipitation of the globulin fractions yielded precipitates with a high Bayer 205 content. The conclusion is reached, therefore, that the drug combines with, or is specifically adsorbed by, some of the plasma proteins.

No effort has so far been made to determine the distribution of the drug between the different plasma proteins, but investigations along these lines are now being made. The results recorded in this paper, and others which will be reported later, indicate that there is no special affinity between fibrinogen and Bayer 205 under the conditions of our experiments. Fibrinogen, precipitated from Bayer 205-containing plasma as fibrinogen or as fibrin, contains a small amount only of the drug, and it is possible that part of this small quantity is associated with non-fibrinogen constituents of the precipitate. Perhaps more conclusive, however, is the fact that plasma and serum derived from the same Bayer 205-containing blood contain exactly the same amount of the drug; from this it can be concluded that no appreciable amount of the drug is attached to the fibrinogen, unless it is assumed that any Bayer 205-fibrinogen complex is broken down when fibrinogen is converted into fibrin to form a clot. Since the latter possibility appears rather unlikely, these results suggest that the anticoagulant action of Bayer 205 is not due simply to its combination with fibrinogen. Further studies are being made to determine which constituents of the blood-clotting system combine with this drug, and it is hoped that further information will also be obtained with regard to the mechanism of the anticomplementary action of the drug.

SUMMARY

1. Investigations have been made to determine the cause of the long retention of Bayer 205 (or Antrypol) in the animal body after injection of the drug. In particular, the possibilities of (*a*) storage in certain organs, and (*b*) combination with plasma- and tissue-proteins have been studied.

2. There is no marked storage of the drug in the liver, heart, muscle, lung, brain, adrenals or pancreas of rabbits which have received one, two or three injections of Antrypol. Each of these organs, except the brain, retains a small but measurable amount of the drug, possibly in combination with the tissue proteins.

3. The kidneys of these injected rabbits contain considerably more Antrypol than do the other tissues examined, and the amount present (in terms of mg. drug/g. kidney) varies appreciably from one animal to another. This retention of the drug in the kidney is of special significance in view of the fairly frequent occurrence of albuminuria following the injection of the drug into man.

4. The spleen contains a little more of the drug than do the other organs examined, but considerably less than the kidney. Slight retention in the spleen might possibly be due to association of the drug with the reticulo-endothelial system.

5. Various protein fractions have been separated from the serum and plasma of injected rabbits, and from normal serum to which Bayer 205 has been added. Evidence has been obtained that plasma globulin and probably other proteins can combine (by adsorption or otherwise) with this drug.

6. The conclusion is reached that the long retention of Bayer 205 in the animal body is due to the combination of the drug with plasma and tissue proteins.

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