rabbit the pigment within one hour can be demonstrated in the blood and urine.

Animal experiments are now under way to study the toxicity and effectiveness of this antibiotic.

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## THE Rh AND Hr FACTORS IN CHIMPANZEES[1]

THE purpose of this paper is to report the results of tests for the Rh blood types and Hr factor on ten chimpanzees, three jungle-born and seven colony-born.

In the Rh and Hr tests,[2] the bloods of all ten chimpanzees behaved alike. In the tests for the Rh blood types, with sera anti-$Rh_0$, anti-Rh′ and anti-Rh″, the reactions were either negative or weak. When any agglutination occurred, this proved to be due to heteroagglutinins rather than specific Rh agglutinins, as was proved by absorption tests. Thus, absorption of the sera with chimpanzee blood removed the agglutinins for chimpanzee blood without affecting the reactivity of the serum for Rh-positive human blood; while absorption with human Rh-positive blood removed the agglutinins for human blood without affecting the reactions of the sera with chimpanzee blood. These results, therefore, indicate that all ten chimpanzees are Rh negative.

That this conclusion is correct was proved by tests for the Hr factor. In tests with an exceptionally potent anti-Hr serum it was found that the chimpanzee bloods were all agglutinated strongly and to the same titer of the serum (about 250) as human Rh-negative blood. Absorption with chimpanzee blood removed the agglutinin for human Rh-negative blood as well as the reaction for chimpanzee blood and, conversely, absorption with human Rh-negative blood destroyed the reactivity of the Hr serum for chimpanzee blood. Moreover, the anti-Hr agglutinin was absorbed equally well by equivalent volumes of chimpanzee and human Rh-negative red cells.

These investigations are being continued, and additional chimpanzees at the Yerkes Laboratories will be tested.* Most likely, the other chimpanzees will also give reactions corresponding to the human Rh-negative type. Perhaps this uniformity in the reactions of chimpanzee bloods is the final result of the selective action of isoimmunization in pregnancy, without the interference of racial crossing such as is apt to occur in man.[3]

[1] Aided by a grant from the United Hospital fund of New York City.

[2] For technique see: A. S. Wiener, J. P. Zepeda, E. B. Sonn and H. Polivka, *Jour. Exp. Med.*, 81: 559, 1945.

* After this article was submitted for publication, blood from five additional chimpanzees was tested, with similar results in the Rh and Hr tests.

[3] A. S. Wiener, SCIENCE, 96: 407, 1942.

In conclusion it should be mentioned that nine of the chimpanzees gave reactions corresponding to group A, while one gave reactions corresponding to group O. This agrees well with previous reports on a total of 92 chimpanzees, of which 81 belonged to group A and 11 to group 0.[4] The bloods of all ten chimpanzees reacted strongly with our anti-M serum, in conformity with the previous finding that all chimpanzees possess M-like agglutinogens.[5, 6] The anti-N serum which we had available did not agglutinate the chimpanzee bloods, but this does not necessarily contradict the conclusion from tests with other anti-N sera that chimpanzee blood also contains N-like agglutinogens.[6, 7]

The authors wish to express their appreciation to the staff of the Yerkes laboratories for their cooperation in obtaining the blood samples.

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## ACCUMULATION OF DDT IN THE BODY FAT AND ITS APPEARANCE IN THE MILK OF DOGS[1]

THE high lipoid-water distribution ratio of DDT suggested that it might be preferentially stored in the adipose tissues of mammals fed DDT. The toxicological behavior of this compound pointed also to possible deposition in body fat. Such a preferential distribution was first indicated by feeding the dibrom analogue of DDT, 2,2-bis(p-bromophenyl)-1,1,1-trichloroethane, to rats and rabbits and determining the increase in tissue levels of bromine. The rise in the bromine content of the fat was many times that in the liver, kidney, brain or blood. These analyses, however, did not show the exact nature of the stored compound. It was not until the specific colorimetric method of Schechter and Haller[2] became available that the material stored in the fat was shown to be the unchanged DDT. The extent to which DDT will accumulate in the fat of chronically fed animals

[4] A. S. Wiener, "Blood Groups and Transfusion," 3rd edition, chapter XIX, C. C Thomas, Springfield, Ill., 1943.

[5] K. Landsteiner and P. Levine, *Jour. Exp. Med.*, 47: 771, 1928.

[6] A. S. Wiener, *Jour. Immunol.*, 34: 11, 1938.

[7] A. S. Wiener, *Am. Nat.*, 75: 199, 1943.

[1] A portion of the funds used in this investigation was supplied by a transfer, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Division of Pharmacology of the Food and Drug Administration.

[2] M. S. Schechter and H. L. Haller, *Jour. Am. Chem. Soc.*, 66: 2129, 1944.

should be an important factor in the toxicological evaluation of this insecticide.

### ACCUMULATION OF DDT IN THE BODY FAT

We had available for this study a number of dogs which had been receiving daily doses of DDT for periods of time varying from 138 days to two years. Since it was desired to continue the chronic experiments, these animals were anesthetized by intravenous injection of 40 mg/kg of sodium pentothal and samples of fat were taken from the peritoneal cavity under aseptic conditions.

The samples of fat were extracted with ether and the DDT determined by the Schechter and Haller[2] method. The quantities found in relation to dosage level, length of administration and form of dosage are shown in Table 1.

TABLE 1

ACCUMULATION OF DDT IN THE BODY FAT OF DOGS

| Dog no. | Sex | Weight kg | Daily dose mg/kg | Form of administration | Days duration | DDT in fat mg/gm |
|---|---|---|---|---|---|---|
| M–166 | f | 8.9 | 10 | soln. | 747 | 0.080* |
| 81–196 | m | 10.0 | 10 | soln. | 747 | 0.024 |
| 1–20 | f | 6.5 | 50 | soln. | 443 | 1.65 |
| 81–195 | m | 10.4 | 50 | soln. | 747 | 4.94 |
| 1–35 | f | 6.9 | 80 | solid | 443 | 0.39 |
| M–171 | m | 10.3 | 80 | solid | 443 | 0.67 |
| After discontinuing dose for 81 days | | | | | | |
| 1–59 | f | 7.3 | 80 | soln. | 138 | 0.013 |
| 1–61 | m | 9.3 | 80 | soln. | 138 | 0.00 |

* For purposes of comparison, the intravenous lethal dose of DDT is of the order of 0.04 milligrams per gram body weight.

Examination of these data reveals several significant facts. Storage of DDT in the body fat increases with level of administration. The fat accumulation is also profoundly influenced by the physical state of the DDT given. The toxicity observed in dogs with these dosages is similarly affected. No dogs, for example, have died from the 80 mg/kg/day dose of the dry solid DDT out of four, whereas the two dogs, 1–59 and 1–61, are the only ones of 16 that survived the 80 mg/kg/day dose of DDT dissolved in corn oil. One of these, we believe, would have also died had the dosage not been discontinued.

The fact that DDT disappears from the fat upon discontinuation of administration is demonstrated by examination of the data obtained in the case of the last two dogs in the table. Supporting evidence for appreciable fat storage in these animals at the time the treatment was withdrawn is the observed continuation of excretion of DDT metabolites in the urine of number 1–59 for 24 days and of number 1–61 for 16 days.

The distribution of DDT between subcutaneous fat and intraperitoneal fat was found to be equal in dog No. 1–35. This observation would indicate that the material is distributed uniformly throughout the fat depots in the body.

### APPEARANCE OF DDT IN THE MILK

A third dog, 1–36, belonging to the group receiving 80 mg/kg/day solid DDT, had just weaned a litter during the course of this experiment. A small sample of milk was obtained upon each of two successive days and analyzed. DDT was found in amounts of 0.06 and 0.04 milligram per gram of milk, respectively.

A control dog, D–3, also with a litter was given a single 50 mg/kg dose of the ortho-para isomer of DDT, 2-o-chlorophenyl, 2-p-chlorophenyl-1,1,1-trichloroethane. Twenty hours later a sample of milk was obtained and found to contain approximately 0.05 milligram of the o,p-isomer per gram.

### CONCLUSIONS

DDT in quantities of significance in its toxicological evaluation is stored in the body fat of dogs given daily oral doses. The storage increases with dosage level. Feeding oil solutions of DDT gives greater accumulation in the fat than does feeding the undissolved material. The accumulated DDT gradually disappears from the fat after discontinuation of administration.

The milk of lactating dogs receiving DDT or its ortho-para isomer contains appreciable levels of the respective compounds.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A NEW TEST FOR BLOOD ESTROGEN

IT is well known that the vagina of the rat remains closed until sexual maturity but opens earlier in response to the administration of estrogens. The test here reported depends upon this phenomenon, but administration is made locally and in the 21-day rat is by this method positive to extremely small dosage.

The procedure is extremely simple. The sample, in