the possibility that other dietary factors are involved.

Summary. When hatching eggs laid by Rhode Island Red hens fed a diet deficient in vitamin B_{12} were injected with crystalline vitamin B_{12}, the hatchability was improved. Among the chicks hatched from vitamin B_{12}-injected eggs, the growth rate was greater, the mortality lower and the feathering better than among chicks hatched from H_2O-injected and non-injected eggs.

Received October 26, 1949. P.S.E.B.M., 1949, v72.

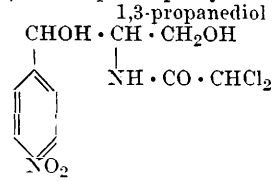
Distribution of Chloramphenicol (Chloromycetin*) and its Metabolic Products Between Human Red Cells and Plasma. (17512)

ANTHONY J. GLAZKO, LORETTA M. WOLF, AND WESLEY A. DILL.

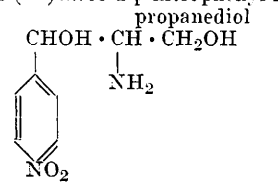
From the Research Laboratories of Parke, Davis and Company, Detroit, Mich.

Following the administration of chloramphenicol to human subjects, 3 aromatic nitro compounds were identified as excretory products in the urine.(1) These were unchanged chloramphenicol (I), an amino diol produced from chloramphenicol by hydrolysis (II), and a conjugate of chloramphenicol with glucuronic acid (III):

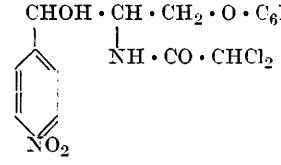
I. Chloramphenicol
D(—)*threo*-1-p-nitrophenyl-2-dichloracetamido-1,3-propanediol



II. Hydrolysis product
D(—)*threo*-1-p-nitrophenyl-2-amino-1,3-propanediol



III. Glucuronide derivative.



Of these compounds only I showed a significant degree of antibiotic activity. The glucuronide derivative III constitutes the major excretory product in human urine, although small amounts of I and II are also present.(1,2) The reduction of the nitro group is negligible in man, although considerable quantities of aryl amines may appear in the urine and feces of lower animals.(2) The data presented here summarize our observations on the distribution of I, II and III between the red cells and plasma of heparinized human blood.

Procedure. The chloramphenicol used in these experiments was a crystalline product isolated from fermentation sources.† The amino diol was prepared by hydrolysis of chloramphenicol,(3) and the glucuronide derivative was isolated from human urine in this laboratory.(1) Blood drawn from the antecubital veins of normal human subjects was treated with a few milligrams of powdered heparin (110 units per mg) to inhibit coagulation, and was used immediately. In a typi-

* Parke, Davis and Company trademark for chloramphenicol.

1. Glazko, A. J., Dill, W. D., and Rebstock, M. C., *J. Biol. Chem.*, 1950, in press.

2. Glazko, A. J., Wolf, L. M., Dill, W. D., and Bratton, A. C., *J. Pharm. Exp. Therap.*, 1949, v96, 445.

† The chloramphenicol used in these experiments was kindly supplied to us by Dr. John Ehrlich and Dr. Fred Stimpert. The amino diol (II) was prepared from chloramphenicol by Dr. Mildred Rebstock and Dr. Harry M. Crooks.

3. Rebstock, M. C., Crooks, H. M., Controulis, J., and Bartz, Q. R., *J. Am. Chem. Soc.*, 1949, v71, 2458.

TABLE I.
Recovery Values for Known Amounts of Chloramphenicol, a Hydrolysis Product, and a Glucuronide-derivative of Chloramphenicol Added to Human Blood.

Compound	Conc. in blood (μg/ml)	Whole blood 1:20 (%)	Whole blood 1:40 (%)	Red cells 1:20 (%)	Red cells 1:40 (%)	Plasma 1:20 (%)	Plasma 1:40 (%)
I. Chloramphenicol	80	81	92	75	87	90	94
II. Hydrolysis product	53	90	96	94	96	94	94
III. Glucuronide derivative	125	89	90	89	95	92	99

Dilutions of 1:20 and 1:40 were made in 3% trichloracetic acid. The filtrates were analyzed for nitro compounds by the titanium reduction procedure. Values in the body of the table are given as per cent recovery, compared with those from aqueous solutions taken as 100%.

cal experiment a solution of purified I, II or III in physiological saline was added to heparinized blood and incubated at 38° with occasional shaking. Aliquots were withdrawn after 30 and 60 minutes, and a portion of each sample was centrifuged at 2100 r.p.m. for 15 minutes to separate the plasma and formed elements. The whole blood, plasma and red cells were then analyzed separately for aromatic nitro compounds, using the colorimetric procedure described elsewhere.(4) This method depends on reduction of the nitro group with titanous chloride, followed by diazotization and coupling with the Bratton-Marshall reagent.(5) The amount of color formed was found to be inversely proportional to the molecular weights of I, II and III. Samples were deproteinized with 3% trichloracetic acid using a sample dilution of 1:40. Colorimetric standards were set up with the same solutions used for the experiments, and corrections were made for recovery from whole blood, plasma and red cells as described in the next section. Packed cell volumes were determined on the original blood specimens and on diluted blood taken at each sampling period. This was done by centrifuging in Wintrobe tubes at 2500 r.p.m. for 15 minutes, reading cell volumes, and then recentrifuging to check the first reading. These figures were used for calculating the total content of nitro derivatives in the plasma and cells as a check on the analytical results.

Results and discussion. Data for the colorimetric recovery of known amounts of I, II and III added to whole blood, plasma and red cells are presented in Table I. The blood samples were diluted 1:20 and 1:40, using a final concentration of 3% trichloracetic acid, and filtrates were analyzed for nitro compounds by the titanous reduction procedure.(4) From these results it is evident that dilutions of at least 1:40 must be used for satisfactory recovery of nitro compounds in the presence of red cells. These values were used to correct the analytical data obtained in the distribution experiments. The distribution of I between red cells and plasma was studied by the addition of 1925 μg I in 6 ml of saline to 10 ml of heparinized blood. After standing at 38° with occasional shaking for 30 and 60 minutes, samples were removed for analysis as already described. In a similar manner, the amino diol compound (II) was studied by the addition of 640 μg II in 3 ml saline to 10 ml of blood from a different individual. The glucuronide derivative was also handled similarly, with the addition of 2325 μg III in 3 ml saline to 10 ml of heparinized blood. The results of these experiments are presented in Table II.

From these data it is evident that I and II are bound to a considerable extent by the formed elements of blood, while III is located almost entirely in the plasma with little or none present in the red cells. The binding is probably due to adsorption rather than slow diffusion through the cell membrane, since higher concentrations of nitro compounds are found in the red cells than would be expected on the basis of uniform distribution. This is also supported by evidence for the binding of

4. Glazko, A. J., Wolf, L. M., and Dill, W. D., *Arch. Biochem.*, 1949, v23, 411.

5. Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, 1939, v128, 537.

TABLE II.
Distribution of Nitro Compounds Between Plasma and Red Cells.

Compound	Theoretical conc. (μg/ml)	Incub. (min.)	Packed cell vol. (%)	Conc. of nitro compounds: Plasma (μg/ml)	Red cells (μg/ml)	Whole blood (μg/ml)	Ratio: red cells/ plasma
I. Chloramphenicol	120	30	32.6	100	153	123	1.53
		60	32.6	98	157	118	1.60
II. Amino diol hydrolysis product	49	30	31.1	39	61	47	1.56
		60	33.7	33	68	46	2.06
III. Glucuronide derivative	179	30	39.3	274	55	168	0.20
		60	40.6	290	38	172	0.13

Plasma, red cells and whole blood were analyzed for nitro compounds following equilibration at 38° for 30 and 60 minutes, as described in the text. Values were corrected for recovery from each fraction as indicated in Table I. The ratios of concentrations found in red cells and plasma are given in the last column.

I to serum albumin.(6) In most cases, the amount of drug accounted for by the separate analysis of plasma and cells fell within 5 per cent of the actual amount of drug added. The observations presented here may assist in the interpretation of published data on the renal excretion of chloramphenicol and its inactive metabolic products.(2) The excretion rate for the glucuronide derivative III is no doubt greater than if it were bound to the cellular elements of blood, while the excretion of unchanged chloramphenicol is probably retarded by adsorption onto the erythrocytes, which would lower the effective concentration of freely diffusible drug in the plasma. The binding of chloramphenicol on the red cells, and possibly on the cells of other tissues, may constitute a reservoir of antibiotic which is in equilibrium with the body fluids. The existence of a different mechanism for producing active chloramphenicol by enzymatic hydrolysis of the inactive glucuronide has been described elsewhere.(2) These two factors may well be responsible for the maintenance of therapeutic levels of chloramphenicol in the blood stream over long periods of time.

Summary. Chloramphenicol and one of its hydrolysis products appear to be partly bound to the cellular elements of blood, while the glucuronide derivative of chloramphenicol remains almost entirely in the plasma. Observations are also presented on the recovery of these aromatic nitro compounds from trichloracetic acid filtrates of plasma, cells and whole blood by colorimetric methods.

6. Vandenbelt, J. M., cited by Smith, R. M., Joslyn, D. A., Gruhzit, O. M., McLean, I. W., Penner, M. A., and Ehrlich, J., *J. Bact.*, 1948, v55, 425.

Received October 27, 1949. P.S.E.B.M., 1949, v72.

A Simple Means of Producing Obesity in the Rat. (17513)

DWIGHT J. INGLE.

From the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

The effects of overfeeding and of obesity have been little studied in the laboratory. Voluntary hyperphagia in rats has been stimulated by insulin(1) and by lesions in the hypothalamus.(2) In this laboratory, we have produced marked obesity in rats by

1. MacKay, E. M., and Cullaway, J. W., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 406.

2. Hetherington, A. W., and Ranson, S. W., *Anat. Rec.*, 1940, v78, 149.