serum to Ehrlich's ascites tumor developed hemorrhagic edematous lungs with a markedly increased serotonin content. Such lung findings were not seen in mouse anaphylaxis.

The authors express their appreciation for the technical assistance of John Donch.

1. Redfern, W. F., *Am. J. Hyg.*, 1926, v6, 276.

2. Cailleau, R., Costa, F., *J. Nat. Cancer Inst.*, 1961, v26, 271.

3. Kind, L. S., *Bact. Rev.*, 1958, v22, 173.

4. Shore, P. A., Parkhurst, A., Burkhalter, A., Cohn, F. J., *J. Pharmacol. Exp. Therap.*, 1959, v127, 182.

5. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Undenfriend, S., *ibid.*, 1956, v117, 82.

6. Treadwell, P. E., Wistar, R., Rasmussen, A. F., *J. Immunol.*, 1960, v84, 539.

7. Humphrey, J. H., Mota, I., *Immunol.*, 1959, v2, 31.

8. Tanaka, N., Leduc, E., *J. Immunol.*, 1956, v77, 198.

9. Stern, K., Davidsohn, I., *ibid.*, 1956, v77, 305.

10. Skillen, R. G., Thienes, C. H., Cangelosi, J., Strain, L., PROC. SOC. EXP., BIOL. & MED., 1961, v107, 178.

11. Stone, C. A., Wenger, H. C., Ludden, C. T., Stavorski, J. M., Ross, C. A., *J. Pharmacol. Exp. Therap.*, 1961, v131, 73.

Received September 5, 1961 P.S.E.B.M., 1961, v108

Excretion into Bile of Intrinsic Factor and Its Vit. B_{12} Complex Following Intravenous Injection. (27051)

KUNIO OKUDA

Department of Medicine, Yamaguchi Medical College, Ube, Japan

It has been shown *in vitro* (1,2,3) that uptake of cyanocobalamin (B_{12}) by liver tissue is enhanced by intrinsic factor concentrate (IF), but whether this phenomenon has any bearing on the biological function of naturally occurring IF is not known. We demonstrated that $Co^{60}B_{12}$, when premixed with IF and injected to rats intravenously, was taken up by the liver in almost absolute preference to other organs (4). It was also shown that degree of the preferential uptake might be related to purity of IF but not to the abundance of reticuloendothelial cells, and that formation of an IF-B_{12} complex was prerequisite to this phenomenon. Our observation has been confirmed by others (5,6). Since IF used in these studies was of hog origin and therefore a heterologous protein to the animals, interpretation of the results seems to be limited because of the possibility of nonspecific uptake of such proteins by the liver. Our further attempt to clarify the mechanism by which the liver captures IF-B_{12} complex has disclosed that part of the complex is immediately excreted into bile following the trapping by the liver, and that the same is true of IF without B_{12}. These findings will not only throw light on the mode of action of IF in hepatic uptake of B_{12}, but will provide a clue as to the mechanism of disposal of foreign substances by the liver. Such data are reported here.

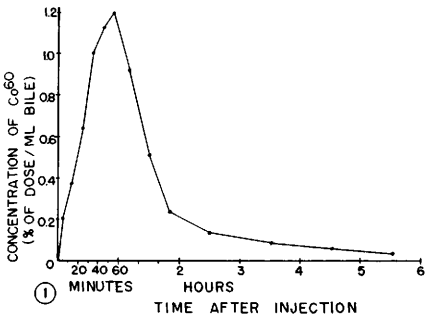
Materials and methods. Young adult rats of Wistar strain which had been raised on a stock diet and rabbits of about 2 kg that had been fed a soy-bean curd diet were used. The IF used was a hog stomach preparation (WES #727) *, and the activity was such that 1 to 2 mg was active in pernicious anemia patients when administred together with 15 μg of B_{12}. The B_{12} binding capacity of this preparation was 500 mμg/mg as measured by dialysis method. The $Co^{60}B_{12}$ used had a specific activity of approximately 0.8 μcurie per μg. Rats were anesthetized with ether and intraperitoneal injection of urethan, 1 ml per 100 g body weight; no anesthesia was given to rabbits. Laparotomy was performed and a glass cannula was inserted into the common bile duct and was tied with the duct. Bile was collected directly from the cannula in the case of rats, or through a polyethylene tubing in the case of rabbits. After securing the basal bile

* Kindly supplied by Dr. L. Ellenbogen, Lederle Laboratories, Pearl River, N. Y.

for half an hour, a saline solution of IF or saline mixture of IF and $Co^{60}B_{12}$ was injected intravenously, the tail vein being used in the former and the ear vein in the latter. The amounts of IF and $Co^{60}B_{12}$ to be mixed were so adjusted that the binding power of IF was always in excess of $Co^{60}B_{12}$ in order to have $Co^{60}B_{12}$ all in bound state.

Aliquots were dried in planchets and counted in a γ-scintillation counter with a flat crystal of NaI. Under the conditions used, 1 mμg of $Co^{60}B_{12}$ gave a count of about 80 above the background of 140 per minute. The binding powers of IF and bile specimens were determined after exhaustive dialysis against large volumes of distilled water for 48 hours, and the basal bile served as the blank. The details of the technique for B_{12} binding measurement and the exhaustive dialysis have been described(4). Some of the bile specimens containing radioactivity were also subjected to equilibrium dialysis. In this dialysis, 2 ml of bile in a Visking bag were dialyzed against 30 ml of distilled water under constant agitation for 48 hours, and both the inside and the outside of the bag measured for radioactivity.

Results. A mixture of 2 mg of IF and 500 mμg of $Co^{60}B_{12}$ was injected intravenously to the rabbit, and bile was collected every 10 minutes for the first hour, every 20 minutes for the next hour and hourly thereafter. The volume held by the cannula and the tubing was allowed to drain before actual timing was started. Concentrations of radioactivity measured in bile clearly demonstrated that excretion of Co^{60} began almost immediately following the injection and reached the peak between 50 and 60 minutes, decreasing sharply thereafter (Fig. 1).



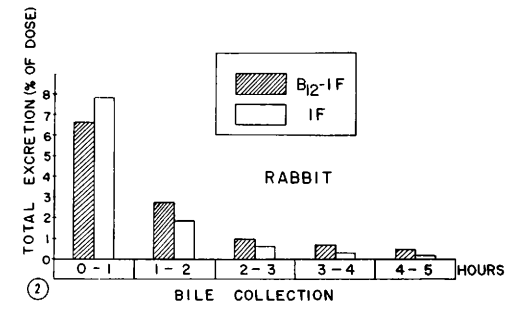


FIG. 1. Rate of Co^{60} excretion in bile following intravenous injection of IF−$Co^{60}B_{12}$ complex to the rabbit. A mixture of 2 mg of IF and 500 mμg of $Co^{60}B_{12}$ was administered. Figures are average of 2 rabbits.

FIG. 2. Total biliary excretion of IF and IF−B_{12} complex following intravenous administration to the rabbit. Shaded bars represent radioactivity excreted in bile following injection of a mixture of 2 mg of IF and 500 mμg of $Co^{60}B_{12}$; open bars represent B_{12} binding power excreted following injection of 2 mg of IF alone. Figures are average of 3 animals.

Under the same condition, bile was accumulated in hourly portions and subjected to exhaustive and equilibrium dialyses to determine the quantitative relationship between bound and free Co^{60}. The results (Table I) showed that most of the radioactivity in the 1st and the 2nd bile specimens remained in

TABLE I. Dialysability of radioactivity excreted in bile following intravenous injection of IF-$Co^{60}B_{12}$ to rabbit.

Bile specimen (Time of collection)	Vol. (ml)	Radioactivity (as mμg of $Co^{60}B_{12}$) Exhaustive dialysis Before (per ml bile)	Exhaustive dialysis After (per ml bile)	After equilibrium dialysis* Inside (2 ml bile)	After equilibrium dialysis* Outside
0-1 hr	9.5	3.7	3.6	7.3	0
1-2 "	7.5	2.0	2.0	4.1	0
2-3 "	6.4	.8	.8	1.5	0
3-4 "	6.9	.5	.4	1.0	0
4-5 "	5.8	.4	.4	.8	0

Two ml of bile used for dialysis, and duplicates were made wherever volume permitted.
* Inside—2 ml bile, outside—30 ml distilled water.

the cellophane bag after dialyses. Since the basal bile contained a B_{12} binding power equivalent to 1.5 mμg/ml, the undialyzed radioactivity in the 3rd, 4th and 5th specimens do not necessarily indicate the presence of an exogenous B_{12} binder. Thus, it occurred to us that Co^{60} excreted in the 1st and 2nd specimens might represent $Co^{60}B_{12}$ still bound to IF. The following experiment was carried out to clarify this point.

Four rabbits were divided into 2 groups, and a mixture of 2 mg of IF and 500 mμg of $Co^{60}B_{12}$ was injected intravenously to one group, and the same dose of IF without B_{12} to the other group. The bile was accumulated in hourly portions, and total radioactivity of each specimen was measured in the former and B_{12} binding power in the latter. The results (Fig. 2) clearly demonstrated that following intravenous administration of IF, there was a marked increase of B_{12} binding power in the bile during the first one hour. Noteworthy also was that the amount of binding power measured in the 1st one-hour specimen of the second group was very similar to the amount of radioactivity of the corresponding bile specimen of the 1st group, if both were expressed in terms of mμg of B_{12}. It seems likely therefore, that the B_{12} binding power excreted in the bile following intravenous administration of IF represented the original IF or IF in an altered form but still retaining the B_{12} binding property. It follows further that the radioactivity excreted in the 1st group also represented the original or a slightly modified IF-B_{12} complex.

A similar experiment was carried out with rats. Six adult female rats were divided into 2 groups, one group receiving intravenously a mixture of 1 mg of IF and 100 mμg of Co^{60}-B_{12}, the other group receiving the same dose of IF alone. Again, a significant amount of radioactivity or B_{12} binding power was measured in bile during the first hour. Furthermore, the quantitative relation between the radioactivity in bile of the 1st group and B_{12} binding power of the 2nd group was practically the same as that found with rabbits.

Discussion. The preferential hepatic uptake of intravenous IF-B_{12} complex first detailed by the author(4) may or may not be related to the natural function of IF. It can be argued that possible pinocytosis of IF molecules by the liver reticuloendothelial system may be responsible as is the case with antigenic heterologous proteins. Nevertheless, it is of interest to postulate the presence of a B_{12} carrier in the blood which is IF itself or behaves like it and has a strong affinity to the liver. As already pointed out, intravenously administered IF-B_{12} complex is not taken up by the spleen or any other organ which is rich in the reticuloendothelial system except the liver. The results of this study have provided further support to this hypothesis by demonstrating that both IF and IF-B_{12} complex are immediately excreted in bile without evidence of extensive chemical changes, following intravenous administration. If the uptake of IF or its B_{12} complex were executed by pinocytotic activity of the Kupffer cells, no explanation seems feasible for the demonstrated instantaneous transfer of the test material from the blood stream into bile, because of the anatomy of the lobule. It is only fair to presume that IF or IF-B_{12} is trapped in the Disse's space which is formed by the microvillous surface of the polygonal cells and the trabeculae of the Kupffer cells, and some of the trapped molecules immediately move from there into the bile canaliculi. The only possible route for such rapid excretion might be the electronmicroscopic gap between the polygonal cells which connects the Disse's space to the bile canaliculus(7). If all the IF molecules are to be engulfed by the Kupffer cells like certain colloid particles, it will take some time before they are ejected from the cells and excreted into bile. Inference may be made, therefore, that IF or IF-B_{12} complex has a strong affinity to the surface of the Disse's space. Such affinity may be due to the presence of a chemical acceptor on the cell surface. The hypothetical carrier of B_{12}, which was simulated by IF in this experiment, may be circulating in blood in minute quantities to regulate the hepatic uptake of B_{12}. A recent study by Brody *et al.*(8) of the kinetics of intravenously injected B_{12} in man has suggested a similar possibility.

Summary. Intrinsic factor or a mixture of IF and $Co^{60}B_{12}$ was injected intravenously to rabbits and rats in which a biliary fistula had been prepared, and excretion of B_{12} binding power or Co^{60} in bile was measured. It was

found that significant amounts of B_{12} binding power or Co^{60} appeared in the bile within one hour and that such excretion began almost immediately following intravenous administration. A possible mechanism of hepatic uptake and subsequent excretion into bile of intravenously administred IF or its complex has been discussed.

1. Miller, O. N., Hunter, F. M., PROC. SOC. EXP. BIOL. & MED., 1958, v97, 863.
2. Latner, A. L., Raine, J. L., *Biochem. J.*, 1957, v66, 53P.
3. Herbert, V., *J. Clin. Invest.*, 1958, v37, 646.
4. Okuda, K., Wider, J. A., Chow, B. F., *J. Lab. & Clin. Med.*, 1959, v54, 535.
5. Troncale, F. J., Hansen, H. J., Miller, O. N., *Fed. Proc.*, 1961, v20, 450.
6. Toporek, M., *Am. J. Physiol.*, 1961, v200, 557.
7. Ashworth, C. T., Sanders, E., *Am. J. Pathol.*, 1960, v37, 343.
8. Brody, E. A., Estren, S., Wasserman, L. R., *Blood*, 1960, v15, 646.

Received September 5, 1961 P.S.E.B.M., 1961, v108

Influence of Spermine and Related Substances on Susceptibility of *Bacillus anthracis* to Lysozyme.‡ (27052)

GEORGE G. WRIGHT*
The Sir William Dunn School of Pathology, University of Oxford

In complex media certain strains of *Bacillus anthracis* become susceptible to the action of lysozyme only when bicarbonate is present during growth(1). This requirement for bicarbonate was not demonstrable in chemically defined media; under these conditions the strains remained susceptible to lysozyme when bicarbonate was omitted. Investigation of the cause of this difference revealed that the yeast extract present in complex media contained a dialyzable factor that caused susceptible strains to become resistant. It was the activity of this factor that was overcome by addition of bicarbonate to the growth medium(2).

Identification of the factor in yeast and elucidation of its action are of particular interest because of the possibility that related mechanisms may be involved in the bicarbonate requirements for elaboration of capsular polypeptide and protective antigen by this organism(3,4). The present report presents evidence that spermine, spermidine, and certain diamines reproduce the effect of yeast extract in inducing resistance to lysozyme.

‡ The author is indebted to Dr. H. H. Johnston for helpful discussion. The work was carried out during tenure of a Secretary of the U. S. Army Research and Study Fellowship.

* Permanent address: Fort Detrick, Frederick, Md.

Methods. The basal medium contained 2% Difco vitamin-free casamino acids, 0.04% Difco yeast extract, and 0.2% glucose. The small amount of yeast extract was sufficient to allow growth of the test organism in the absence of lysozyme but not in its presence. The medium was adjusted to pH 7.6 with sodium hydroxide and sterilized by filtration. Ultra-fine sintered glass filters were used for all sterilizing filtrations. Sterile sodium bicarbonate solution was added to the basal medium in the concentrations indicated.

The compounds tested were obtained from commercial sources, spermine and the diamines as hydrochlorides, spermidine as the phosphate, and agmatine and arcain as sulphates. They were dissolved in water and sterilized by filtration. Titrations were carried out in ¾" x 6" tubes provided with aluminum caps. Serial 2-fold dilutions of the substances tested were prepared in a volume of 0.5 ml. To each tube were added 0.5 ml of 1-1000 solution of crystalline lysozyme (Armour Laboratories) and 1.0 ml of basal medium. Controls without lysozyme, without test substance, and without inoculum were included in each titration.

The inoclum consisted of approximately 100 spores of strain B of *B. anthracis*, added