tained from cord specimens than from specimens from normal adults(2). This has been confirmed in the present studies. Elution rate has been shown to be directly related to percent fetal hemoglobin present. Rapid rates of elution were also present in hemoglobin solutions containing fetal hemoglobin obtained from patients with Cooley's anemia. Elution from intact erythrocytes has previously not been investigated. In the present studies no clear cut difference in elution could be demonstrated between labeled intact red blood cells from adults, infants, and patients with Cooley's anemia.

Elution of chromium from normal erythrocytes has been shown to occur at a level of approximately 1% per day(5-9). It would be difficult to demonstrate a significant difference in rate of elution from erythrocytes of normal adults and from cells containing large percentages of fetal hemoglobin by dialysis where the error of the method may be of the same order of magnitude as the possible difference in rate of elution. Evidence thus far does not support the existence of any influence by fetal hemoglobin on rate of chromium elution from intact erythrocytes. A final solution to this problem might be obtained by studies of survival of erythrocytes from patients with Cooley's anemia in normal recipients simultaneously by the differential agglutination and chromium^{51} technics.

Summary. Accelerated rates of chromium^{51} elution from hemoglobin solutions containing fetal hemoglobin have been shown to be present in specimens obtained from an infant and from patients with Cooley's anemia. This acceleration has been shown to be directly related to the percent fetal hemoglobin present. Rapid rates of chromium^{51} elution could not be demonstrated to occur from intact labeled erythrocytes containing large amounts of fetal hemoglobin studied by the technic of dialysis.

1. Hellingsworth, J. W., *J. Lab. and Clin. Med.,* 1955, v45, 469.
2. Suderman, H. J., White, F. D., Israels, L. G., *Science,* 1957, v126, 650.
3. Weinstein, I. M., Spurling, C. L., Klein, H., Necheles, T. F., *Blood,* 1954, v9, 1155.
4. Erlandson, M. E., Schulman, I., Stern, G., Smith, C. H., *Pediatrics,* in press.
5. Gray, S. J., Sterling, K., *J. Clin. Invest.,* 1950, v29, 1604.
6. Ebaugh, F. G., Emerson, C. P., Ross, J. F., *ibid.,* 1953, v32, 1260.
7. Necheles, T. F., Weinstein, I. M., LeRoy, G. V., *J. Lab. Clin. Med.,* 1953, v42, 358.
8. Reilly, W. A., French, R. M., Lau, F. Y. K., Scott, K. G., White, W. E., *Circulation,* 1954, v9, 571.
9. Read, R. C., *New Eng. J. Med.,* 1954, v250, 1021.
10. Kaplan, E., Zuelzer, W. W., *J. Lab. Clin. and Clin. Med.,* 1950, v36, 511.

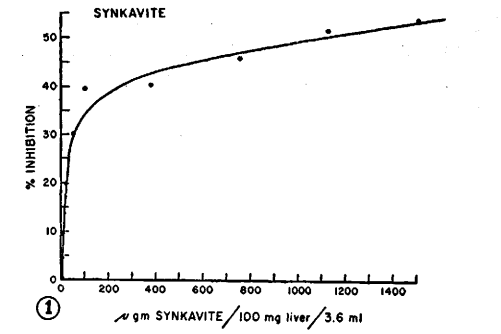
Received July 15, 1958. P.S.E.B.M., 1958, v99.

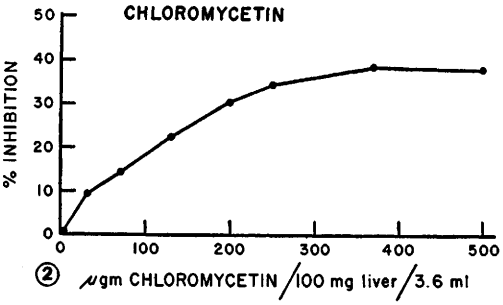
Inhibition of Bilirubin Conjugation *in vitro.** (24285)

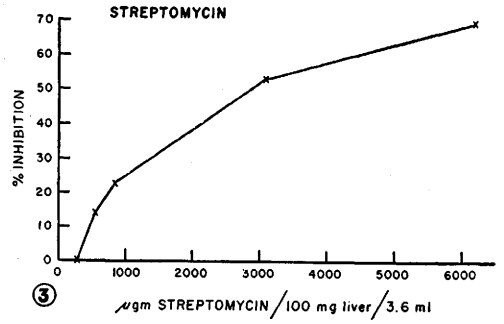
WILLIAM J. WATERS, ROBERT DUNHAM, AND WILLET R. BOWEN
(Introduced by L. K. Diamond)
Department of Pediatrics, State University of New York College of Medicine at Syracuse

In recent years we have been interested in the problem of bilirubin metabolism in the newborn and the mechanism of production of bilirubin encephalopathy as well as its prevention(1). Recent studies have suggested an impairment of substrate production and conjugation of bilirubin as a glucuronide in the newborn period(2). In the light of this knowledge, the reports of jaundice associated primarily with 30 mg or more of Synkavite in 2-3 kg newborn infants(3,4) stimulated us to assess the possible role of the latter in inhibition of the conjugation mechanism leading to hyperbilirubinemia. Recent reports would also suggest another possible mechanism of action, namely, increased hemolysis of newborn red cells(5). The report of Silverman *et al.*(6), further stimulated us to test a num-

* Supported by Grant from the N.I.H. of the P.H.S.







GRAPH 1. % inhibition of bilirubin conjugation in presence of increasing concentrations of synkavite.

GRAPH 2. % inhibition of bilirubin conjugation in presence of increasing concentrations of chloromycetin.

GRAPH 3. % inhibition of bilirubin conjugation in presence of increasing concentrations of streptomycin.

ber of antibiotics and chemotherapeutic agents regarding the possible role of these compounds in the conjugation mechanism.

In vitro studies were carried out using procedures identical to those previously published by others. The preparation and treatment of the liver extracts and homogenates were as outlined by Dutton and Storey(7). The diazoatation was a modification by Lathe (personal communication) of the van den Bergh reaction.

Our objective was to determine the amount of conjugated bilirubin produced in a given incubation and possible inhibition of conjugation as a result of the presence of these potential inhibitors. The amount of conjugated bilirubin in 2 runs was determined, one with and one without the competing substrate. Otherwise, standard conditions were maintained in the 2 flasks and the variation in amount of conjugated bilirubin produced on incubation was assumed to be due to the potential inhibitors.

In all compounds investigated at least 2 series were run to determine the inhibition curves. Reproducibility of inhibition in any series using the same inhibitor and different rats' liver as a substrate and enzyme source was not possible. However, usually the variation did not exceed 10% and this was assumed to be due to varying concentrations of substrate and enzyme in the individual rat liver.

In the newborn, one might expect serum concentrations of at least 20-40 μg/ml of chloromycetin and streptomycin in the usual dosage range (Wehrle, personal communication).

Results. The effect of Synkavite is shown in Graph I. There is an immediate sharp rise in inhibition with a slight increase beyond 250 μg. Initial inhibition is detected at 5 μg and the curve flattens out at 1500 μg.

The effect of chloromycetin is shown in Graph II. The curve represents an average obtained in 2 runs. Initial inhibition is detected at 30 μg with some increase up to 250 μg with a subsequent stabilization.

The effect of streptomycin is shown in Graph III. The curve represents an average obtained in 2 runs. Inhibition is first detected at 560 μg with a fairly steep rise at very high concentrations.

Penicillin, tetracycline, erythromycin, gantrisin, and hydrocortisone show essentially no inhibition in the concentrations investigated which are listed in Chart #1.

Conclusion. Since serum levels are unknown in relation to Synkavite, the data are difficult to interpret but it would appear that inhibition *in vitro* occurs at concentrations which

CHART I. Non-Inhibitors.

Compound	Conc./flask
Penicillin G	0–400 μ
Tetracycline	0–400 μg
Erythromycin	0– 1 mg
Gantrisin	0– 16 mg
Hydrocortisone	0–500 μg

might be expected in an infant, particularly when high doses are given. Chloromycetin shows slight inhibition within the accepted range and streptomycin just beyond the accepted range of serum levels.

1. Waters, W. J., *J. Ped.*, 1958, v52, 559.
2. Lacson, P. S., Waters, W. J., *Am. J. Dis. Child. Soc. Trans.*, 1957, v94, 510.
3. Crosse, M. V., Meyer, T. C., *ibid.*, 1957, v90, 602.
4. Allison, A. C., *Lancet*, 1955, v1, 669.
5. Zinkham, W. H., Childs, B., *Am. J. Dis. Child. Soc. Trans.*, 1957, v94, 420.
6. Silverman, W. A., Andersen, D. H., Blanc, W. A., Crozier, D. N., *Pediatrics*, 1956, v18, 614.
7. Dutton, I. D. E., Storey, G. J., *Biochem. J.*, 1954, v57, 275.

Received July 18, 1958. P.S.E.B.M., 1958, v99.

Inhibition of Diaminopimelic Acid Decarboxylase Activity in *Mycobacterium tuberculosis* by Isonicotinic Acid Hydrazide.* (24286)

HILDA POPE WILLETT (Introduced by D. T. Smith)
Department of Bacteriology, Duke University Medical Center, Durham, N. C.

In an attempt to determine the mechanism of action of isonicotinic acid hydrazide (INH) on *Mycobacterium tuberculosis* attention has been directed in r any studies toward an interference with the operation of essential enzymes. Inhibition of growth by INH can be neutralized competitively by pyridoxal, which the drug structurally resembles(1,2). A number of enzyme systems requiring pyridoxal phosphate as coenzyme have also been shown to react with INH(3-5). Although these studies suggest an antagonism, there is as yet no direct evidence that INH exerts its antituberculous activity by inhibiting an enzyme activated by pyridoxal phosphate.

In a recent study in our laboratory it was found that growth of tubercle bacilli in the presence of INH leads to a marked decrease in amount of lysine produced, and that lysine synthesis by the INH-resistant strain is also lower than that by the INH sensitive organism. Since one of the major pathways of lysine synthesis in microorganisms is by way of the pyridoxal phosphate-requiring enzyme, diaminopimelic acid (DAP) decarboxylase, we have investigated the possibility that this enzyme is operative in tubercle bacilli and that it is inhibited in the presence of INH.

Methods. The RlRv strain of *Myco. tuberculosis,* kindly furnished by Dr. William Steenken, was used as the source of enzyme in all of our studies. Cells were harvested after a 3-week growth period on the surface of Proskauer-Beck synthetic liquid medium. They were washed twice in a refrigerated centrifuge with sterile distilled water, resuspended in a small amount of 0.1 M phosphate buffer and ground in a Ten Broeck tissue grinder. Additional buffer was added, the cells recentrifuged, and suspended in a known quantity of buffer. The cells were disrupted in a Raytheon 9KC sonic oscillator followed by centrifugation at 16,000 x G for 30 min. The straw-colored supernatant contained the active enzyme and was used immediately. All operations were carried out in the cold and because of the long reaction time employed, sterile conditions were maintained throughout all the experiments.

The reaction mixture for detecting DAP decarboxylase activity contained 12.5 μM synthetic DAP, 10 μg pyridoxal phosphate,

* This study was aided by a grant from the Committee on Medical Research of the American Trudeau Soc., medical section of the National Tuberculosis Assn.