increased in relation to age in intact growing, and in early and late hypophysectomized rats. 2. Statistical analysis of values obtained shows that regression coefficients of Age vs. EST of intact growing and of late hypophysectomized rats are almost identical, whereas those of early hypophysectomized rats are lower. It is possible that early hypophysectomized rats become more sensitive to the stimulus than intact or late hypophysectomized animals. 3. The conclusion is reached that other variables, particularly age, have to be considered in seeking information of the effect of deficiency state itself on EST, or more generally on central nervous system sensitivity. On this basis, many previous conclusions require revision.

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Studies in Histochemistry LIII. Microbiological Assay in Quantitative Histo- and Cytochemistry.* (24454)

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Quantitative chemical analysis of histologically or cytologically defined samples often requires micro technics to deal with the small quantities involved. Microbiological assay, the method of choice for certain B vitamins etc., has a remarkably great sensitivity which is well suited to the exacting requirements of histo- and cytochemical studies. Nevertheless, this analytical approach has been surprisingly neglected, and in fact only an occasional micro adaptation for any purpose has been made, *e.g.*,(1-4). Bioautography offers a sensitive means of detection but without the degree of quantitation afforded by the usual microbiological assay. Excellent comprehensive reviews of these technics have just appeared(5,6). Recent instrumental developments have made feasible the extension of microbiological assay to a scale requiring 500 to 1000 times less sample without loss in accuracy or precision. In this communication such a technic will be described with application to measurement of biotin, liberated from bound form by acid hydrolysis, and pantothenate, liberated by enzyme hydrolysis.

Materials and methods. The micro equipment employed has been described(7). Pyrex culture tubes 28 mm long, 4 mm inner diameter, with sleeve-type rubber serum-bottle stoppers were used. Constriction pipettes were employed for all volumes under 100 μl and mixing in the tubes was carried out by "buzzing." A constant temperature water bath with a plastic plate containing holes, 6 mm diameter, to hold the culture tubes served as the micro incubator. The plate was suspended 15 mm under the water surface so that the tubes were submerged but the tops of the stoppers were not. An ordinary well-controlled incubator can be used instead. A micro centrifuge (Microchemical Specialties Co., Berkeley) was also used. Growth was determined turbidimetrically at 650 mμ with a Beckman DU spectrophotometer. Lowry-Bessey micro cuvettes were employed for larger volumes (100 μl) and Glick-Grunbaum inserts were used with these cuvettes for small volumes (6 μl). *Lactobacillus arabinosus,* strain 17-5, with "double strength"

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Skeggs-Wright medium was used for assay of biotin(8) and pantothenate(9). For both vitamins substitution of cysteine for cystine and addition of 1 mg folic acid per liter of medium was found to give more consistent results. For inoculum cultures, 0.025 μg % biotin or 2 μg % calcium d-pantothenate was added to the medium diluted with an equal volume of water. Stock cultures were maintained on APT Agar, No. 229 (Case Laboratories, Chicago). When the sample was a microtome section of fresh-frozen tissue, it was frozen-dried at –20°C in a vacuum desiccator over silica gel(7), weighed on a quartz fiber micro balance, and then subjected to hydrolysis. For biotin, optimal conditions for hydrolysis were found to be autoclaving the dried sample, *e.g.*, about 20 μg from a fresh section of rat liver 2 mm diameter and 20 μ thick, in 5 μl of 2 N sulfuric acid in a stoppered tube for 1 hour at 120°C. The hydrolysate was neutralized with 5 μl of 2 N sodium hydroxide and diluted with sufficient (usually 180 μl) 0.015 M phosphate buffer, pH 7, to bring the biotin concentration into the region required by a standard calibration curve. For pantothenate in the dried sample, *e.g.* liver as above, enzyme hydrolysis, adapted from the procedure of Novelli and Schmetz(10), required 18 hours at 37°C in a mixture consisting of 5 μl (2 Schmidt-Thannhauser units) of 0.2% purified intestinal alkaline phosphatase (Pentex, Kankakee, Ill.), 10 μl of 10% suspension of Dowex-treated pigeon liver acetone powder (Pentex) in 0.02 M potassium bicarbonate, 5 μl of 0.1 M Tris buffer, pH 8.4, and 30 μl water. The enzymes were inactivated by immersing the tubes in boiling water for 45 seconds and the hydrolysate then diluted with 100 μl of water. Before assay the hydrolysates of both vitamins were clarified by centrifugation for several minutes at 7500 g or greater. Sterile standard solutions of biotin in 0.015 M phosphate buffer, pH 7, were used in the range 0.1-1.0 mμg per ml. The calcium d-pantothenate standards were in the range of 10-100 mμg/ml water. Aliquots of 10 μl of standard or hydrolysate solution were employed for each assay, and these were set up in triplicate for each dilution. Hydrolysates were diluted 2 or 3 times and all dilutions were assayed simultaneously. Ten μl of inoculated medium were added to each tube and the mixture incubated at 30°C for 24 hours (pantothenate) or 48 hours (biotin). Finally 100 μl of 0.9% sodium chloride were added, the solution mixed and the absorbance measured.

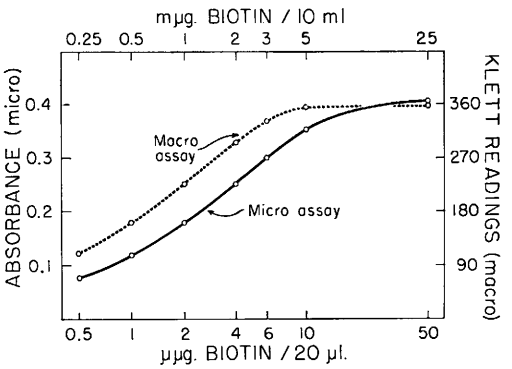

FIG. 1. Response of *Lactobacillus arabinosus* (17-5) to biotin in macro (10 ml/tube) and micro (20 μl/tube) assays. Bacterial suspension was not diluted before measurement in the former, but it was (1:1) in the latter.

Results. The data presented in Fig. 1 illustrate the close correlation between the macro and micro assays for biotin. The same degree of correlation obtains for pantothenate assays. To test the precision of the methods 10 aliquots from a standard solution of biotin and 10 from one of coenzyme A were analyzed and the respective means 4.05 $\mu\mu$g (% standard deviation and error, 3.2 and 1.0) and 22.6 mμg (% standard deviation and error, 4.9 and 1.5) were obtained. Triplicate analyses of hydrolysates of individual microtome sections of rat liver, dry weight 21-24 μg, gave a mean of 3.14 $\mu\mu$g biotin per μg dry weight for 10 sections (% standard deviation and error, 4.4 and 1.6) and 0.28 mμg pantothenic acid per μg dry weight for 7 sections (% standard deviation and error, 7.9 and 2.8).

Preliminary work indicates that the method for biotin can be used for niacin in the range 0.006-1 mμg by replacing the niacin in the double-strength medium by 0.1 μg % biotin. Hydrolysis requires autoclaving for 15 minutes at 120°C in 0.1 N sulfuric acid; 0.1 N sodium hydroxide is used for neutralization.

Summary. Extension of microbiological as-

say methods to a scale requiring 1/500th to 1/1000th the sample ordinarily used is described with specific application to the measurement of biotin and pantothenate in tissue sections.

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Antimycobacterial Activity of Extracts of Rat Peritoneal Cells.* (24455)

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Lurie concluded (a) that acquired immunity to tuberculosis is merely enhanced natural resistance, and modes of action of the 2 phenomena are probably the same(1,2) and (b) that altered physiological reactivity of mononuclear phagocytes of the immunized animal is the most important single factor in resistance to the disease(3). These conclusions have gained considerable support. It appeared possible therefore that comparative studies of some physiological activities of mononuclear cells from animals of differing susceptibility to this disease might contribute to a better understanding of mechanisms of restriction of intracellular mycobacterial growth. As a part of such studies, antimycobacterial activities of extracts of cells of induced peritoneal exudates from the highly susceptible guinea pig and the very resistant rat have been investigated. It seems desirable to report preliminary results of these experiments.

Materials and methods. Male albino guinea pigs of the "English" breed weighing 800 to 1200 g each, and 2 strains of male albino rats of 250 to 400 g weight were used in groups of 8 to 18 animals for each experiment. Exudate induced by intraperitoneal injection of glycogen(4) was collected on sixth day, at time of monocyte-type cell predominance, by washing peritoneal cavities with large quantities of isotonic 0.005 M Tris buffer, pH 7.4. Fluid was collected by gravity from living guinea pigs, and from rats by exposing peritoneal cavities immediately following their death from sharp head blows. Siliconed glassware or lusteroid or polyethylene containers were used for handling all cell suspensions. About 1 to 2.5 l of pooled cell suspension was obtained from each group of animals. The number of cells was determined by hemocytometer for preliminary standardization. Cells were concentrated in 20 to 40 ml of isotonic Tris buffer in a refrigerated centrifuge. Concentrated cell suspensions were freed from contaminating erythrocytes, if any, by recentrifuging and suspending 10 minutes in hypotonic NaCl solution, which was then made isotonic by addition of an equal volume of hypertonic NaCl solution(5). Total and differential cell counts were made and the suspension recentrifuged; thus cells were washed twice after removal from animals. The button of cells was evenly suspended in glass-distilled water to make concentrations of about 60 to 70 x 10^6 cells/ml and suspensions were subjected to sonic vibration in Raytheon

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