pletely protected against the spread of the challenge inoculation, and 4 in each group (18.2%) presenting only localized tuberculous lesions and the remaining 5 and 6 animals (25%) showing advanced and generalized tuberculous lesions. Table I shows that neither group of animals immunized with irradiated vaccine showed an animal completely protected against the challenge inoculation, that 9 and 10 animals (54.3%) presented localized tuberculous lesions and the remaining 8 animals in each group (45.7%) showing advanced and generalized tuberculosis.

The quantitative data on enlarged regional lymph glands, the pooled total 17 sets of routinely dissected lymph nodes, and on the spleen, liver and lungs from animals immunized with 2 types of irradiated vaccine and BCG vaccine, together with those from the control animals, are given in Table I. These data bear out the gross pathological findings mentioned above, namely that irradiated killed tuberculosis vaccine is surely capable of producing specific resistance against a challenge virulent tuberculous infection. But in comparison with either series of BCG immunized animals, the protective value of living BCG vaccine is significantly greater than that of killed irradiated tuberculosis vaccine. In view of the incomplete immunity produced in animals and in man with the relatively avirulent BCG living organisms, our study suggests that it would be premature to substitute for BCG a vaccine consisting of killed tubercle bacilli.

Summary. Living BCG vaccine has shown superior antigenic potency to killed irradiated tuberculosis vaccines by producing significantly greater tuberculin hypersensitiveness and specific resistance against a challenge virulent inoculation in guinea pigs.

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Received December 5, 1951. P.S.E.B.M., 1952, v79.

Ionization and Distribution Between Aqueous and Non-Aqueous Solvents of Dyes in Relation to Gastric Secretion.* (19390)

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Recently(1) a formula was devised in this laboratory that correlated the pK_B of certain basic dyes with their tendency to be secreted by the stomach. While successful in leading to the discovery of new compounds that were secreted in substantial amounts, certain discrepancies were evident(2). In the new series only compounds with a pK_B over 10 were secreted while in the original series the optimum was 6.5 to 9. These discrepancies might be caused by the fact that highly purified dyes were used in the original(1) pK_B determinations while Ingraham and Visscher

* Supported by a Grant from the Anna Fuller Fund.

TABLE I. Comparison of pK_B of Pure and Commercial Dyes.

Compound	C.I. No.	Stomach*/Blood	pK_B Pure	pK_B Com'l	Absorption maxima (mμ)
Nile blue sulfate	913	.8	11.6	10.9	640
Acridine red	740	8.4	10.9	5.9	555
Rhodamine B	749	6.1	10.8	10.3	555
Brilliant cresyl blue	877	11.3	10.8	9.4	625
Methylene green	924	13.6	10.8	9.9	620
Neutral violet	826	27.2	10.8	7.4	535
Methylene blue	922	15.6	10.2	—	660
Bismark brown	331	26.5	9	9.2	440
Chrysoidin Y	20	75	8.7	9	455
Safranin O	841	27.3	7.6	—	520
Neutral red	825	26.4	7.5	7.3	530
Thionin	920	36.8	7.1	7.2	600
Toluidine blue	925	36.4	6.5	6.1	625
Pyronine B	741	5.7	6.3	7.1	550

* Ref. 3.

(3) in their determination of the stomach: blood ratios used commercial products. Although there is no certainty that the present commercial dyes are identical with those used by the authors mentioned, it seemed of interest to repeat the pK_B determinations on the commercial products. The weakness of the pK_B determination of polybasic compounds is that it is a stepwise process. It was thought that the distribution between an acid buffer and benzene might be a better measure of total effective basicity and would correspond more closely with the stomach:blood ratios.

Methods. A. Basic ionization constants were determined by potentiometric titration employing the Beckman G, pH meter with glass electrode 1190T and calomel electrode 1170. Beckman electrode 1190E was used when the pH rose above 10. The electrodes were checked in standard buffers before and after each determination. The room temperature was maintained at 25 ± 1°. From 0.1200 to 0.2500 g of dye was dissolved in 300 ml of water and titrated with 0.048N sodium hydroxide. Values were taken from the curves in the usual manner. *B.* Equal volumes (5 ml) of buffer, $NaHSO_4/Na_2SO_4$, pH 2, or KH_2PO_4/Na_2HPO_4, pH 6.73, and benzene were shaken together with the dye until equilibrium was achieved. The two phases were separated and the concentration measured in a Beckman Model DU spectophotometer and compared with a standard. Dyes that were insoluble in the buffer were first dissolved in water and then diluted with buffer solution. By using samples of 1 to 10 μg per ml Beer's Law was valid. Because of the dilute solutions used, Dalton's Law applies and inorganic impurities that may be present do not effect the results. The absorption maxima are listed in the last column of Table I.

Results. A. Basic ionization constants. Most of the pK_B determinations on the impure dyes agree rather well with those reported for the purified substances. In Table I the compounds are arranged in the order of decreasing pK_B (increasing basicity) of the pure compounds. The titration curves of the commercial dyes did not rise as sharply nor were the plateaus as well defined. In fact 2 compounds gave such obscure curves that no definite values could be assigned. Acridine Red seems to be one compound that shows widely differing values: 10.9 for the pure and 5.9 for the impure. Its transposition to the bottom of the Table would serve to place it just as satisfactorily on the descending end of the curve. Neutral Violet on the new basis would also fall into a more favorable position next to Safranin O which has a similar stomach:blood ratio. Pyronine B, however, with a stomach:blood ratio of 5.7 would now be located between Thionin and Toluidine Blue, both of which have stomach:blood ratios of about 36. Thus the position of most compounds is unaffected; 2 compounds are better placed; while one is misplaced by the data

TABLE II. Distribution Ratios (Commercial Dyes).

Compound	C.I. No.	pH buffer	Buffer / Benzene	Stomach / Blood
Nile blue sulfate	913	2	2.7	.8
Chrysoidin Y	20	2	4.3	75
Bismark brown	331	2	5.7	26.5
Neutral violet	826	2	‡	27.2
Neutral red	825	2	"	26.4
Toluidine blue	925	2	"	36.4
Nile blue sulfate	913	6.73*	§	.8
Bismark brown	331	6.73	"	26.5
Chrysoidin Y	20	6.73	"	75
Neutral violet	826	6.73	.68	27.2
Pyronine B	741	6.73	1.96	5.7
Methylene green	924	6.73	2.5	13.6
Brilliant cresyl blue	877	6.73	2.7	11.3
Toluidine blue	925	6.73	2.9	36.4
Safranin O	841	6.73	5.6	27.3
Thionin	920	6.73	11.5	36.8
Methylene blue	922	6.73	‡	15.6

* Neutral Red, Acridine Red and Rhodamine B became colorless when shaken with benzene buffer at pH 6.73. † Ref. 3. ‡ 100% in buffer. § 100% in benzene.

on commercial dyes. The stomach:blood ratio for Chrysoidin Y is decidedly out of line with all other values and must be presumed to be in error. The new dyes of the fluorene series (2) were found not to be secreted by the stomach of the rat at a pK_B as low as 8.5. It is obvious that the results on the commercial dyes do not throw any light on this. In fact, by changing the value of Neutral Violet from 10.8 to 7.4 it definitely takes the one good analogous value out of consideration.

B. Distribution ratios. In Table II the compounds are arranged in the order of decreasing concentration in the benzene layer (order of increasing basicity). Two different buffers were used, pH 2 and 6.73. Three of the compounds, Acridine Red, Rhodamine B and Neutral Red became colorless or yellow when shaken with the benzene-buffer at pH 6.73. On evaporation of the benzene the red color returned. Probably the free bases are colorless and the compounds are thus unsatisfactory for this type of investigation. Nile Blue becomes red because of oxazone formation. It too, is unsuitable. In column 5 of Table II, the stomach:blood ratios are those given by Ingraham and Visscher(3). Column 4 gives the values for the buffer:benzene. In considering the first series measured at pH 2 the distribution ratios parallel pK_B values for the commercial dyes. The pK_B determination for pure Neutral Violet is again less satisfactory than that of the commercial dye. The stomach:blood ratios also fall fairly well on a plateau for compounds with a buffer:benzene ratio of 5.7 to infinity. The stomach:blood ratio for Chrysoidin seems to be entirely out of line with all other values and can only be accepted with reservation. Experiments in this laboratory, however, show that its secretion is at least as great as that of Neutral Violet.

Since half of the dyes went 100% into the buffer at pH 2, it was decided to increase the pH in order to secure a better evaluation. The results are shown for pH 6.73 in the lower section of Table II.

Here the 3 compounds that gave finite values for the distribution ratio at pH 2 were completely in the benzene layer but only one, Methylene Blue, was entirely in the buffer. A careful inspection of the data at pH 6.73 reveals no relation between the distribution ratios and either the stomach:blood ratios or the pK_B values of pure or commercial dyes. A pH of 6.73 is thus definitely unsuitable for this type of investigation. A value intermediate, say pH 4, would result, however, in other compounds besides Methylene Blue being entirely in the buffer layer.

Summary. In a study of the relation of physical chemical properties of dyes to the

extent of their secretion by the stomach, new determinations of the pK_B values and absorption maxima were made. A somewhat better correlation of pK_B and stomach:blood ratios was obtained by using commercial instead of pure samples of dyes. The distribution of the dyes between benzene and aqueous buffers of pH 2 and pH 6.73 was measured but no satisfactory correlation found. The method is limited by the tendency of compounds to dissolve 100% in either the buffer or the benzene.

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Received January 3, 1952. P.S.E.B.M., 1952, v79.

Inactivation of 11-Dehydro-17-Hydroxycorticosterone by Tissue Slices.* (19391)

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Numerous investigations carried out during the past several years have clearly demonstrated that the liver is the organ most concerned with the inactivation of estrogens, testosterone and desoxycorticosterone(1-3). However, when 11-dehydro-17-hydroxycorticosterone (cortisone) became available for study, it became apparent that degradation of such 11-oxygenated steroids might differ from that of estradiol, testosterone and desoxycorticosterone. The latter steroids are practically ineffective when given orally, whereas cortisone§ is approximately 80% as effective orally as parenterally. The following data however, strongly suggest that the 11-oxygenated steroids are rapidly inactivated by the body. A very small percentage of administered cortisone can be recovered from the urine of the patients. The finding of relatively high concentrations of 11-oxygenated steroids in the adrenal vein blood, and of very little in peripheral blood(4,5) is also suggestive of inactivation. deAndino, Fontan, and Pashkis (6) showed that intrasplenic injection of cortisone was ineffective in decreasing thymic weight unless huge doses were administered, thus implicating the liver in the inactivation of this steroid.

The purpose of this study was to determine which organs of the body might inactivate cortisone *in vitro.* In addition, experiments were devised to ascertain whether the inactivation mechanism could be modified.

Methods and materials. Rat tissue slices taken from normal male rats immediately after decapitation were used for studies of cortisone inactivation. Liver, kidney, spleen, muscle, and brain were sliced by hand; 300 to 350 mg of tissue were incubated in 5 ml of Krebs-Ringer solution (pH 7.4) with 100 to 250 μg of cortisone for 3 hours at 37°C. After incubation, the tissue slices were homogenized in a Ten Broeck homogenizer, kept at 4°C overnight and the whole homogenate bioassayed on the following morning as described below. Two types of control were used: 1) tissue slices which had been boiled previously for 10 minutes; 2) non-incubated controls to which the cortisone was added immediately before bioassay. This was performed to exclude the possibility of adsorption of the steroid to the tissue protein. Cortisone, in the form of the free alcohol, was prepared in 95% ethanol in the concentration of 1 mg/ml and 0.1-0.25 ml of this standard solution were

* Aided by grants from the American Cancer Society as recommended by the National Research Council, Committee on Growth and the Ciba Pharmaceutical Co.

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§ Kindly supplied by Merck, Inc.