terial deaminase by exposure to molar phosphate buffer at pH 4 is not yet understood, is not always consistent, and doubtlessly is influenced by a variety of factors, these data afford confirmation that biotin somehow is concerned in activation of the system. Further study of the variables involved during inactivation and reactivation of the deaminase system is indicated before final conclusions can be drawn concerning the significance of biotin in these reactions.

*Summary.* Inactivation of bacterial aspartic acid deaminase activity by exposure of cells to pH 4 M phosphate buffer and its reactivation with biotin has been confirmed.

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## Binding of Phenolsulfonephthalein by Tissues and the Effect of Denaturation Thereon.* (17497)

WINTHROP FISH, GEORGE H. MILLER AND CHARLES HUGGINS

*From the Department of Surgery, University of Chicago, Chicago, Ill.*

The present experiments were conducted to study (1) the binding of the organic anionic dye phenolsulfonephthalein (PSP) by homogenates of several tissues of the rat; (2) the effect of denaturation of tissue proteins on this binding; and (3) the competitive effect of native and denatured tissues on the binding of PSP by crystallized bovine plasma albumin. It has been previously established that certain proteins can form complexes with organic anions on both the acid and basic side of the isoelectric point. De Haan(1) and Marshall and Vickers(2) demonstrated that the anion PSP was bound appreciably by serum proteins and Grollman(3) showed that at the pH of normal blood the binding was primarily by serum albumin, the globulins binding very little dye. Klotz and Urquhart(4) showed that anions are also bound to $\beta$-lactoglobulin, hemocyanin and $\alpha_2$ globulin, although to a much smaller extent than occurs with serum albumin. Ohta(5) working with tissue brei preparations presented evidence that both anionic and cationic dyes were adsorbed by tissue proteins. Denaturation of serum albumin by heat(6,7) or urea(7) abolishes the binding capacity for oleate and methyl orange anions. A different effect occurs with egg albumin in that heat denaturation increases the binding of alkylbenzenesulfonate detergents(8) and methyl orange.(7)

*Experimental.* Albino rats were exsanguinated by cardiac puncture or decapitation and weighed samples of the tissues were homogenized for approximately two minutes in M/15 phosphate buffer, pH 6.5. In the experiments, measured volumes containing 250-500 mg of the original tissue together with 1.0 ml of a solution of PSP in phosphate buffer pH 7 were placed in a cellophane bag 15 cm long and 1.5 cm in diameter. The samples were dialyzed at 5°C against M/15 phosphate buffer pH 6.5 in flasks which were slowly rotated for 40 hours to reach equilibrium; the total volume of the system was 100 ml. The bound PSP was calculated from the concentration of free PSP in the dialysate

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as determined colorimetrically by means of a photoelectric colorimeter with a 540 millimicron filter. The results were expressed in terms of the amount of dye bound by 400 mg of tissue, wet weight. In experiments with crystallized bovine plasma albumin (Armour) the protein was dissolved in M/15 phosphate buffer, pH 7.4, and used in a concentration of 5 g per 100 ml.

Tissues were denatured by the following methods (1) Heat: Tubes containing the tissue were placed in a bath of boiling water for 30 minutes; (2) Contact with urea in 6 Molar final concentration for three hours: The urea mixtures were placed in cellophane bags 30 cm in length and dialysed against distilled water for 5 days at 5°C to remove the denaturing agent. Aliquots containing 250 mg of tissue were used and the results were calculated on the basis of 400 mg of tissue. The effect of the tissues on the binding of PSP by albumin was determined by adding samples containing 400 mg of tissue to a mixture of 1.0 mg of PSP and 50 mg of albumin. The mixture was dialysed against M/15 phosphate buffer at pH 6.5. M/15 phosphate buffer was used for control of pH in all experiments and the pH of all solutions was determined with a glass electrode at the termination of each experiment. This buffer was found sufficient to maintain a constant pH during the course of the experiment. By mixing tissues with PSP and storing at 5°C for 48 hours with sampling at intervals it was found that the tissues employed did not cause a decrease in color of PSP.

*Results and Discussion.* The binding ability of liver, kidney, spleen, testis, pancreas and serum for PSP is shown in Table I. All of the tissues were found to bind the dye, although usually less than serum did; the liver was the most active tissue and pancreas the least. There was found to be some variation in the values obtained in the various experiments although the relative status of the tissues between themselves was maintained. The variation between the binding capacity of the liver of various rats was approximately ± 25%. No doubt this variation was due to the natural differences between the animals used. Denaturation of liver, kidney, and pancreas tissue proteins by heat or urea (Table II) resulted in a striking increase in the binding of PSP by all of these tissues. The increase in the binding by the liver tissue proteins denatured by both agents was of the same magnitude. In contrast to the tissues, denaturation of serum by heat markedly decreased its capacity for dye binding. The effect of denaturation of rat tissue proteins on dye binding is similar to the results obtained with egg albumin. These facts suggest that, in the denaturation process, either more foci of attachment for the PSP are opened in the binding proteins, as is the case with egg albumin, or that hitherto inactive proteins have become capable of binding.

When homogenates of native liver, kidney or pancreas were added to bovine albumin (Fig. 1) the binding of PSP was much less than the arithmetic sum of the dye bound by albumin alone plus the dye bound by the tissues alone. On the other hand no such decrease of binding was observed when heat-denatured tissue homogenates were added to bovine albumin; the mixture bound nearly as much PSP as the proteins did when tested separately. This experiment is interpreted as indicating that binding occurred between the

TABLE I.
Binding of PSP by Native Tissues.

| Tissue | No. of determinations | Range, units |
|---|---|---|
| Serum | 6 | 60-104 |
| Liver | 10 | 30-87.5 |
| Renal cortex | 5 | 20.8-64 |
| Renal medulla | 5 | 29.4-60 |
| Spleen | 3 | 18.4-78 |
| Testis | 6 | 16.1-31.6 |
| Pancreas | 4 | 8-30 |

* The units are micrograms of dye bound by 400 mg of tissue or 0.4 ml of serum.

TABLE II.
Effect of Heat Denaturation on Binding of PSP by Serum and Tissues.

| | Serum | Liver | Kidney | Pancreas |
|---|---|---|---|---|
| Unheated | 62 (4) | 65 (9) | 44 (3) | 30 (3) |
| Heated | 8 (4) | 125 (9) | 75 (3) | 55 (3) |

Figures in brackets indicate the number of animals tested. Average values are given for micrograms of dye bound by 400 mg of tissue or 0.4 ml of serum.

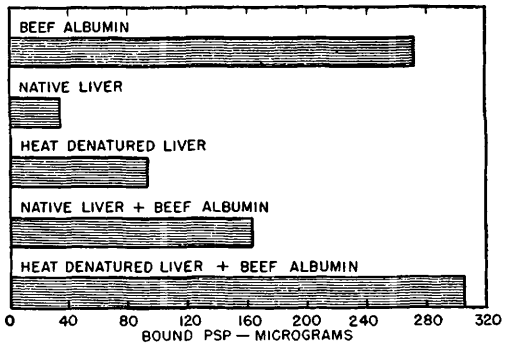


FIG. 1.

Effect of adding native, and heat denatured, homogenized tissue on the binding of phenolsulfonephthalein by crystallized bovine plasma albumin, 50 mg.

tissue proteins with the albumin molecule thus decreasing the available points of attachment for PSP in the system while heat denaturation of the tissue proteins decreased their attraction for albumin increasing the amount of dye anions bound by the mixture.

*Summary.* 1. Tissue proteins are capable of binding the anionic dye, phenolsulfonephthalein, in small amounts. All of the tissues examined bound less of the dye than did serum and different tissues appear to have different capabilities in this respect. Of the tissues tested liver bound most and pancreas least.

2. Denaturation of tissue proteins by heat or urea materially increased the ability of the tissue protein to bind the dye.

3. Native tissue proteins of the rat considerably decreased the binding of phenolsulfonephthalein by crystallized bovine albumin; this property was abolished by heat denaturation of the tissue proteins.

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## Effect of Aureomycin on the Herpes Simplex Virus in Embryonated Eggs.* (17498)

G. DOUGLAS BALDRIDGE AND HARVEY BLANK (With the technical assistance of Ben Kaneda). (Introduced by Werner Henle).

*From the Children's Hospital of Philadelphia and the Departments of Dermatology and Pediatrics, University of Pennsylvania, School of Medicine, Philadelphia, Pa.*

Aureomycin was shown by Wong and Cox to have marked therapeutic activity against the viruses of the psittacosis-lymphogranuloma group.(1) They demonstrated that 49 of 55 treated chick embryos survived experimental infection with psittacosis through the 13th day, contrasted with survival of only 3 of 57 control embryos. These results were found to be essentially true for the other members of the psittacosis-lymphogranuloma group (lymphogranuloma venereum, psittacosis, SF strain human pneumonitis, mouse pneumonitis, feline pneumonitis, and meningopneumonitis F97 strain) as well as for the rickettsiae. In contrast with the marked activity *in vivo,* they conclude that the drug had no *in vitro* effect against these agents. Harned *et al.,* in a study on the acute toxicity of intravenously administered aureomycin hydrochloride for several laboratory animals, found the LD50 for the drug to be 134 mg/kg for mice and 118 mg/kg for rats.(2) Clinical accounts of successful treatment of

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Much of the preliminary work incident to this study was done by Dr. Lewis L. Coriell.

The activity levels for inactivated aureomycin were performed by Dr. Coleman Whitlock.

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