period of time than if the first drug was simply removed and the bacteria permitted to multiply in drug-free broth. That this effect might be attributed to summation (though not synergism) of drug action (perhaps attributable to incomplete removal of the first drug) was suggested by the following experiment. At the time of centrifugation an untreated broth culture was diluted so as to approximate the bacterial concentration of the tube in which the first drug had acted for 4 hours. When a drug was added to this smaller number of untreated bacteria, the rate of multiplication was somewhat more rapid than that of organisms exposed to a sequence of drugs.

This experiment indicates that the additive, though never synergistic effect of two drugs in sequence, can not be attributed to the smaller number of microorganisms acted upon by the second drug. It has not been ruled out that this minor effect could be the result of small, subinhibitory concentrations of the first drug remaining adsorbed on bacteria after washing.

It has been reported that a sudden increase in bactericidal rate followed the addition of streptomycin to an enterococcal population previously exposed to penicillin action for 24 to 72 hours(3). It was similarly observed that in another synergistic drug pair acting on staphylococci, the addition of bacitracin at intervals of 2 to 6 hours to a population exposed to terramycin (or vice versa) resulted in a prompt increase in the rate of bactericidal action. Here, too, even though both drugs were not added initially, simultaneous exposure of microorganisms to both members of a synergistic drug pair resulted in prompt synergism.

Summary and conclusions. (1) It has been demonstrated in two systems that antibiotic synergism is observed only when both members of a synergistic pair of drugs are acting simultaneously on the bacterial population, not when they act in sequence. It may, therefore, be suggested that antibiotic synergism is not usually the result of a modification in a bacterial population induced by one drug, which renders the microorganisms more susceptible to the other drug of a synergistic pair. (2) These findings would tend to favor a hypothesis of synergism involving simultaneous blocking of alternate enzymatic pathways.

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A Quantitative Method for Study of Phenolsulfonphthalein (PSP) Concentration by Surviving Renal Slices.* (19202)

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The prevailing method(1) for studying the transport of phenolsulfonphthalein (PSP) by renal tubular cells *in vitro* is a visual one. The concentrations of PSP, substrates or inhibitors are varied and the ratio of concentrations of any two of these variables required to produce an all or none effect (visible concentration or no concentration) is noted by microscopic examination. The technic employed by Cross and Taggart(2) to investigate the tubular transport of p-aminohippurate (PAH) by surviving renal cortical slices of the rabbit suggested that a similar procedure might be used in *in vitro* studies on the renal tubular transport of PSP, providing a satisfactory method were available for the quantitative estimation of this substance in

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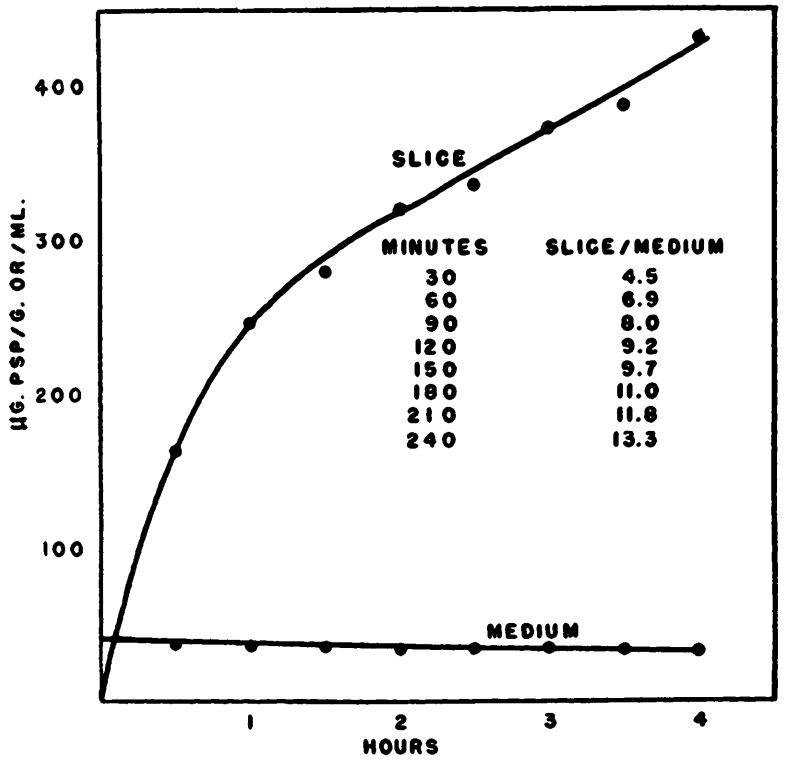

FIG. 1. Effect of incubation time on uptake of PSP by guinea pig kidney slices.

the tissue. Such a method would allow for accumulation of considerably more information with expenditure of less effort than that involved in the technic presently employed.

A technic, described previously in a preliminary report(3), was devised for the estimation of PSP in renal slices and although this yielded satisfactory results it was rather time consuming. A more rapid method designed for this determination is reported herewith.

Methods. Kidneys from guinea pigs were employed in all experiments. The animals were killed by a blow on the head, the kidneys removed, stripped of their capsule and placed in an ice-cold medium of the same composition as that in which they were subsequently incubated, except that the PSP was omitted. Each kidney was quartered and sliced as described by Umbreit, Burris and Stauffer(4), only 3 slices being prepared from each quarter. The slices were then placed in 20 ml beakers containing 5 ml of the buffered medium described by Cross and Taggart(2) and containing 1.06×10^{-4} M (4 mg %) PSP instead of PAH. Care was taken to distribute the slices such that an equal number from each slice level was placed in each beaker. The beakers were placed in a Dubnoff metabolic shaking incubator and shaken at about 100 cycles per minute in an oxygen atmosphere at 25°C. The incubation time was 1 hour and the tissue weight approximately 100 mg unless otherwise stated. After incubation, the beakers were placed in an ice bath and as rapidly as possible the slices removed, blotted and weighed on a torsion balance. For the determination of PSP concentration in the slices, the weighed tissue was transferred to a Potter-Elvehjem homogenizer(5) and homogenized in 11 ml of distilled water. The proteins were precipitated from a 10 ml aliquot of this homogenate by the addition of 1 ml of 10% sodium tungstate followed by 1 ml of 0.67 N sulfuric acid. After mixing and centrifuging, the supernatant was decanted and the precipitate washed twice with 5 ml portions of distilled water. The supernatant and washings were combined and evaporated to dryness in a boiling water bath.

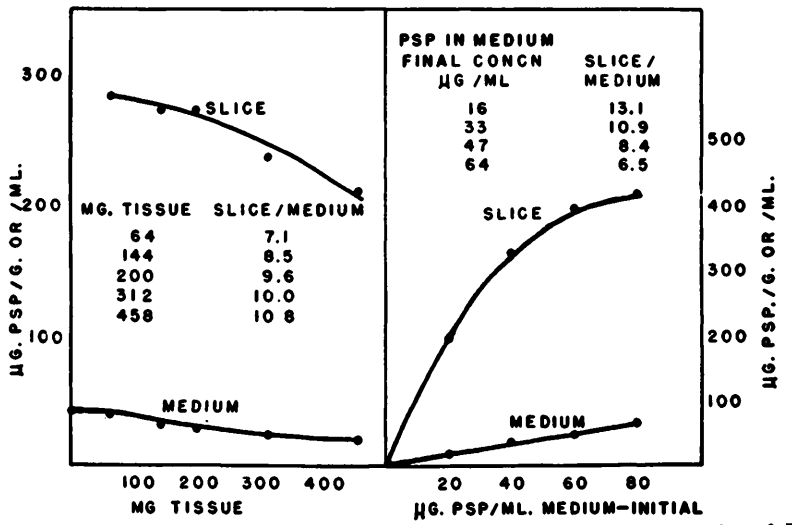

FIG. 2. Influence of tissue wt (left) and PSP concentration (right) on uptake of PSP by guinea pig kidney slices. Along the ordinates are plotted final concentrations of PSP per g of slice or per ml of medium.

Ten ml of 0.1 N sodium hydroxide was added to the dry residue and the concentration of PSP determined in a Beckman spectrophotometer at 564 mµ, using as a reference solution 0.1 N sodium hydroxide. Recovery of known amounts of PSP, added to the renal slices before homogenization, ranged from 97% to 99.5%. After removal of the slices, the medium was centrifuged and 0.4 ml of the supernatant added to 10 ml of 0.1 N NaOH. The concentration of PSP was estimated spectrophotometrically as above.

Results and discussion. In Fig. 1 are portrayed the data illustrating the effect of varying the time of incubation on the uptake of PSP by the renal cortex slice of the guinea pig. The ratio of concentration of dye in the slice to that in the medium (S/M ratio) increased progressively throughout 4 hours. The curve is very similar to that obtained by Taggart(6) for the uptake of PAH from a phosphate buffered medium by the renal slice of the rabbit. Beyer *et al.*(1) have used a bicarbonate buffer for studying the visible concentration of PSP by guinea pig renal slices at 37°C. Employing this bicarbonate buffered medium and a temperature of 37°C we have found that PSP uptake is poor. Maximum concentration occurred in approximately fifteen minutes and on longer incubation the S/M ratio decreased.

The effect of increasing the concentration of PSP in the medium on uptake of dye by the slice is shown in Fig. 2. At low concentrations of PSP in the medium higher S/M ratios are established than at higher concentrations. However, at these low concentrations accuracy of determination of the S/M ratio is sacrificed to some extent.

Cross and Taggart(2) have stated that the amount of tissue employed in their studies on accumulation of PAH by the rabbit kidney slice could be varied between 200 and 400 mg without appreciably affecting the S/M ratio. A similar situation appears to exist when one studies accumulation of PSP by the renal slice of the guinea pig (Fig. 2). Although a progressive increase in S/M ratio is noted as the amount of tissue is increased, above 200 mg this is not great.

Summary. A method has been described for the quantitative extraction and spectrophotometric estimation of phenosulfonphthalein (PSP) in renal cortex slices of the guinea pig. The effect of alterations of time of incubation, concentration of the PSP in the medium, and weight of tissue on the *in vitro* uptake of PSP by this preparation have been investigated. Uptake of PSP increases progressively with increases in incubation time. Increasing the concentration of PSP in the medium results in a decrease in the ratio of

dye concentration in the slice to that in the medium. Ability of the slice to concentrate PSP from the medium is not markedly influenced by alterations of tissue weight between 200 and 400 mg.

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Effect of 6n-prophylthiouracil on Lethal Seizures in Mice.* (19203)

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The antithyroid activity of thiouracil has been often demonstrated and is generally accepted. On the theory that functional inhibition of the thyroid gland would produce decreased spontaneous activity, thiouracil was used to produce a reduction in susceptibility of an inbred strain of mice to sound-induced lethal convulsive seizures.

The author previously found that *age* played an important role in the susceptibility to sound-induced lethal convulsive seizures in tests made on several different inbred strains of mice(1-3). Since the endocrine and physiological levels vary throughout the life span of an individual it was believed that age and metabolism might influence the susceptibility to stress. This was found to be true and, in particular, was demonstrated in the DBA, or inbred dilute brown strain of mice where the highest susceptibility to lethal convulsive seizures at the sound of an electric bell was at the adolescent ages 25-39 days(4). Beyond 80 days of age there were no cases of convulsive individuals. Both sexes were affected, the males slightly more so. The form of convulsive seizures which occur in the DBA strain of mice simulates the grand mal epileptic seizures in humans and it is interesting to note that medical men as Lennox(5) have found the incidence of epilepsy to be greater among children than adults and that the sexes are equally affected. The mouse seizures are of clonic-tonic form and are usually fatal at the peak of susceptibility in the DBA strain.

Methods.† The strain of mice used for these experiments is the dilute brown, DBA/2 Jax, maintained by brother to sister mating. The animals came from the author's colony and are derived from over 200 generations of inbreeding. Two hundred and one animals were used in this experiment: 151 as test or experimental animals and 50 sibs as direct controls. In addition the established performance of the strain as a whole(4) could be used for comparison. Both sexes were used in equal numbers. Seizure susceptibility tests were made at 30 days of age at which age mice are not sexually mature. Thiouracil treatment was started at 21 days of age when the mice weighed about 12 g each. The drug was administered in the food for 9 days previous to the test for seizures, since administering the drug through the drinking water did not prove practicable. The 6n-prophylthiouracil used was obtained from the Overbrook Chemical Co. (Philadelphia). This compound is one of the most highly active antithyroid agents as shown by Astwood(6). Purina Laboratory

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