broth adjusted to pH 6.5. All pH determinations were made with the glass electrode. One hundred cc each of these 4 broths were inoculated with 1 cc each of the same 24-hour broth culture of *Esch. coli,* and incubated at 37°C. Plate counts were made after 24 and 48 hours at this temperature.

Results obtained from studying 13 different commercial pectins of widely varying grades and types showed that pectins *per se* do not exert germicidal action. This does not confirm previously reported findings.[2] In fact in all 13 pectin broths adjusted to pH 6.5 and also in the control broths at the same pH the inoculated *Esch. coli* showed large increases after 24 and 48 hours' incubation. Furthermore in the 9 pectin broths with "natural" pH's ranging from 5.5 to 4.9 and also in the correspondingly adjusted control broths the inocula showed large increases after incubation. On the other hand in the 4 pectin broths whose "natural" pH's ranged from 4.4 to 3.9 marked decreases of the inoculated *Esch. coli* occurred which were paralleled by the counts of the control broths adjusted to these same low pH's. Thus it is seen that H-ion concentration is the factor responsible for these decreases in counts. Additional confirmation of this conclusion is obtained from the pH of the pectin and control broths after incubation. Determination of this pH is necessary to interpret properly the data secured in this type of study. This is specially significant when studying pectins that contain fermentable carbohydrates as "filler", which are used frequently to standardize the grade of the pectin.

10296

Occurrence of Choline-Esterase in Swine.

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The purpose of this study was to find potent sources of choline-esterase which might be obtained conveniently in large amounts for studies on the purification and properties of this enzyme, and to make preliminary observations on its distribution within tissues of

[2] Haynes, E., Tompkins, C. A., Washburn, G., Winters, M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 839.

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a single species in regard to its possible bearing upon nerve physiology.

Heretofore, investigations have been carried out on the relative choline-esterase activities of a given organ[1] or body fluid[2, 3, 4] from various species, and a great variety of papers have appeared dealing with the activity of a certain tissue from a certain species, usually in connection with a definite physiological function. However, very little has appeared in the nature of a survey of the relative activities of the tissues within a given species.

Plattner and Hintner[5] determined, by pharmacological means, the time required for the destruction of 50% of the acetylcholine in digestion mixture by individual organ extracts or body fluids. A single species of animal was not used throughout as a source of material so that strict comparisons between different tissues was not always possible. Furthermore, the method of measurement employed was not very satisfactory since activity is no longer a linear function of time when even much less than 50% of the substrate is hydrolysed in a similar digestion mixture. Quastel, Tennenbaum, and Wheatley[6] measured the choline-esterase activity of slices of several tissues from the rat and guinea pig by the manometric chemical method, as well as the ability of these tissues to produce choline esters *in vitro,* and concluded that there is no correlation between the two. It is interesting to note that Shaw[7] has observed the acetylcholine concentrations in 4 organs of the dog to fall in the order: submaxillary gland > small intestine > stomach > liver, which happens to be the same as the order of the choline-esterase activities found in the present investigation for these organs in swine.

The organs from full-grown animals were removed within 1 hour after slaughtering and transported at once to the laboratory where they were minced in a meat grinder. Five g were weighed out and then subjected to further grinding in a mortar with washed and ignited sand. The disintegrated material was taken up with 10 ml bicarbonate-Ringer solution, centrifuged at a constant speed of about 2500 r.p.m. for 5 min., and the supernatant fluid decanted. In cases where the organ or tissue weighed less than 5 g the cor-

1 Marnay, A., *Compt. rend. soc. biol.*, 1938, **128**, 519.

2 Stedman, E., Stedman, E., and White, A. C., *Biochem. J.*, 1933, **27**, 1055.

3 Stedman, E., and Stedman, E., *Biochem. J.*, 1935, **29**, 2107.

4 Hall, G. E., and Lucas, C. C., *J. Pharm. Exp. Therap.*, 1937, **61**, 10.

5 Plattner, F., and Hintner, H., *Arch. ges. Physiol.*, 1930, **225**, 19.

6 Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Biochem. J.*, 1936, **30**, 1668.

7 Shaw, F. H., *Biochem. J.*, 1938, **32**, 1002.

responding volumes of bicarbonate-Ringer solution were employed. When only small amounts of a tissue were available, or when the tissue could be easily disintegrated as in the case of brain, the initial mincing in the meat grinder was omitted. One-half ml of this fluid was used with 1.5 ml of substrate solution (containing 7.5 mg acetylcholine chloride dissolved in bicarbonate-Ringer solution) for the determination of the enzyme activity by the manometric procedure using the Warburg apparatus.[8] The thermostat was maintained at 30° and manometer readings were taken every 5 min for 30 min.

Activity-time curves were constructed and only values of the linear initial reaction velocities were used. In cases of very great activity the course of the reaction was often no longer linear toward the end of the 30 min period so that it was necessary to extrapolate the linear portion to obtain the 30 min value.

A source of error in comparing the activities of the different tissues lies in the extraction procedure employed. In the ideal case, the enzyme would be completely removed from the tissues so that the extracts would enable comparisons of total activities. Previous experience has shown that choline esterase is readily extracted from small bits of tissue about equally as well by 30% glycerol or physiological saline solution.[9] However, it has been found in this work that a certain amount of the enzyme is retained by the residue after extraction, chiefly due to adsorption. The fineness to which the tissue is ground largely determines the completeness of extraction and since the particle-size undoubtedly varied in different grindings, a source of error was introduced at this point. In addition it has been observed for tissues very rich in choline-esterase that the total activities measured by the procedure described are somewhat less than those found when the extracts are used in greater dilution. In other words, the concentration of enzyme is so great in these cases that there is a deviation from the linear relationship between activity and enzyme concentration which holds for the lower concentrations. Nevertheless, for the purposes at hand the data obtained by employing a uniform extraction technic was sufficient to enable a fairly adequate comparison of tissue activities.

It has been observed that the extracts retain their activities quite well when stored in the refrigerator. Thus an extract of supracervical lymph nodes was found to have an activity corresponding

[8] Ammon, R., *Arch. ges. Physiol.*, 1933, **233**, 486.

[9] Glick, D., *J. Gen. Physiol.*, 1938, **21**, 297; *Compt. rend. lab. Carlsberg*, 1938, **21**, 269.

to the liberation of 28 cmm CO_2 under the conditions employed in these experiments, and after 11 days the activity was unchanged. Another preparation changed from 35 to 31 in 18 days. Similarly an extract of submaxillary gland changed from 194 to 186 in 11 days, and fell to 168 after 7 more days. After 13 days several jejunal extracts remained unchanged.

The data in Table I are self-explanatory. The lack of activity in bile and gall bladder is no doubt the result of inhibiting action of

TABLE I.
Choline-Esterase Activities of Various Swine Organs and Tissues.

Organ or tissue	Enzyme Activity* (mm³ CO_2 evolved in 30 min. at pH 7.4 at 30°C by .5 ml extract)	Avg
Heart	23, 21, 24, 23, 29	24
Skeletal Muscle (Masseter)	25, 25, 26, 27, 26	26
Lung	20, 24, 28, 37	27
Liver	70, 77, 77, 74, 77	75
Spleen	64, 64, 52, 66, 61, 60, 56	60
Kidney	25, 17, 18, 18, 29	21
Pancreas	55, 60, 55, 55, 60	57
Thyroid	21, 23, 27, 32, 27	26
Thymus	30, 22, 29, 30	28
Adrenal	26, 28, 20, 26, 24	25
Testis	67, 67, 69	68
Ovary	45, 68, 53, 45	53
Fallopian Tubes	362, 256, 251, 352, 286	301
Endometrium	90, 83, 91, 87	88‡
Stomach Mucosa (Pylorus)	194, 186, 182	187‡
Duodenal Mucosa	182, 196, 160, 172, 159	174‡
Jejunal Mucosa	190, 198, 165, 200, 210	193‡
Lachrymal Gland	1032, 1320, 1056	1136§
Sublingual Gland (Mixed Mucous and Serous)	1200, 984, 786	990§
Parotid Gland (Serous)	2550, 2340, 2670, 2400, 2460, 2470	2481§
Submaxillary Gland (Mainly Mucous)	181, 220, 158, 223, 153	187
Cerebrum	45, 44, 48, 51, 60	50
Cerebellum	86, 85, 79, 99	87
Medulla Oblongata	130, 146, 163, 134, 117	138
Spinal Cord	81, 74, 81	79‖
Lymph Nodes (Mesenteric)	71, 53, 53, 56	58
" " (Supracervical)	29, 21, 28, 35, 26	28
Lens	12, 6, 9, 15	11
Gallbladder Mucosa	0, 0, 0	0
Bile	0, 0	0
Serum†	9, 7, 7	8

*A separate animal was used as the source of the material for each measurement upon any given organ or tissue.

†0.5 ml of 10% serum used.

‡The mucosa was scraped off, and histological examination showed only traces of muscle tissue.

§Manometer readings taken every 2 min. until top of scale was reached.

‖Thoracic region.

the bile salts.[10, 11] One might expect the very high activity found in the salivary and lachrymal glands to be related to the fact that these tissues respond strongly to cholinergic stimulation.

Summary. The choline-esterase activities of various swine organs and tissues were measured, and particularly high activities were observed in the cases of the lachrymal and salivary glands, the fallopian tubes, alimentary mucosa, and the medulla oblongata.

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Comparative Therapeutic Effects of Sulfapyridine in Experimental *Staphylococcus aureus* Infections in Mice.*

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It has been shown that sulfanilamide has a slight therapeutic effect in the treatment of experimental staphylococcal infections in mice.[1, 2, 3] However, its efficacy has not been great and the drug has been of little value in the treatment of staphylococcal infections in man, in which the tissues or blood stream have been seriously invaded by these organisms. Recently Whitby[4] without giving details of his experiments has shown that sulfapyridine has a definite chemotherapeutic effect in experimental staphylococcal infections in mice.

We have been testing the comparative therapeutic effects of sulfapyridine and sulfanilamide in experimental staphylococcus aureus

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2 Mellon, R. R., Shinn, L. E., and McBroom, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 563.

3 Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

4 Whitby, L. E. H., *Lancet*, 1938, **2**, 1095.