An intestinal extract was prepared by water extraction of the duodenum and first few inches of the ileum of a dog. The extent of digestion was practically identical with that caused by combined action of commercial trypsin and erepsin. The addition of these enzymes produced no further cleavage. No digestion of the carbohydrate portion of the molecule was observed.

These studies indicate that mucin, in contrast to most proteins, is relatively resistant to enzymatic hydrolysis *in vitro*, a property in accord with its ascribed protective action. However, it seems probable that further digestion of mucin occurs in the digestive tract. This is indicated by the fact that normally no readily detectable quantities of mucin are excreted from the intestinal tract, and, furthermore, that glucuronic acid of mucin is available for conjugation.[4] Perhaps specific enzymes exist for this purpose, or some definite but at present unknown sequence of enzymatic action is required. These studies are being continued with purified enzyme preparations.

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Depressor Extracts of Some Human Tissues.*

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The recent work of Dale, Dudley, and their associates[1, 2, 3] has thrown new light on the depressor substances that may be extracted from animal tissues. The methods of preparation of and differentiation between the various depressor substances have been described especially by Chang and Gaddum.[1] Because these methods are rather new, they have not yet been applied extensively to human tissues. The present work was undertaken to determine if there were any unusual amount of depressor substance in (1) carcinomatous tissue and in (2) toxic thyroid tissue.

All specimens were obtained from living patients during a sterile surgical operation. The specimens were extracted in 3 hours or less

[4] Miller, C. O., Brazda, F. G., and Elliott, E. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 633. Miller, C. O., and Connor, J. A., *Ibid.*, 1933, **30**, 630.

* Work done in part under a grant from the Douglas Smith Foundation.

[1] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[2] Dudley, H. W., *J. Physiol.*, 1933, **79**, 249.

[3] Euler, U. S., and Gaddum, J. H., *J. Physiol.*, 1931, **72**, 74.

following operation and were kept in a refrigerator at 4°C. in the meantime. The tissue was weighed and placed in 10% trichloracetic acid (2 cc. of acid for each gm. of tissue). It was then cut up in the acid with scissors and left with occasional stirring for several hours. The extract was filtered through paper on a Buchner funnel and the tissue washed with 7% trichloracetic acid. The filtrate was then shaken 3 or 4 times with ether in a separating funnel until it was only faintly acid to congo red paper. It was then concentrated at low pressure at 37°C. until 1 cc. of extract corresponded to about 1 gm. of tissue. This solution was made neutral to congo red solution by titrating with 1-10 N NaOH and then used in the biological test.

In the present work the extracts were tested for effect on dog's and rabbit's blood pressure. The blood pressure was measured by a mercury manometer connected to a cannula in the right carotid artery. The extract was injected by means of a burette connected with a cannula in the left femoral vein of the dogs or the left external jugular vein of the rabbits. The depressor action was compared with that produced by a standard solution of acetylcholine, made up so that 1 cc. corresponded to 1 gamma acetylcholine. If 1 cc. of the unknown extract gave a fall in blood pressure equal to that produced by 1 cc. of the standard solution, then the unknown extract was said to contain 1γ acetylcholine equivalent per cc. An effort was then made to determine whether or not the depressor action was due to acetylcholine or some other substance. According to Chang and Gaddum, acetylcholine is quite inactive when tested on the blood pressure of an atropinized rabbit. As will be shown later in the present work, most of the tissues extracted produced quantitatively as great a fall in blood pressure of a rabbit after atropinization as before. Control injections of pure acetylcholine in these rabbits produced no fall in blood pressure after atropinization. This indicates that in the extracts tested, using the atropinized rabbit's blood pressure as a criterion, acetylcholine was not present in large amounts.

According to Chang and Gaddum, histamine produces little fall in rabbit's blood pressure. In the present studies the tissue extracts were just as active on the blood pressure of rabbits as on that of dogs, both when compared as to absolute dose per unit body weight and as to acetylcholine-equivalent. This indicates that in the extracts tested, using this criterion of Chang and Gaddum, histamine was not present in large amounts.

Euler and Gaddum[3] found large amounts of a substance they

called the "P" substance in certain animal extracts. This substance lowers the arterial blood pressure of the atropinized rabbit. It can be differentiated from adenosine which is stable in alkalis and inhibits the rabbit's intestine. Wilson, Stewart and Harkins,[4] working in Wilkie's laboratory found that the depressor substance in the skin of burned and normal rabbits answered the specifications of the P substance and that acetylcholine was not present in anything but small amounts.

Results. Six human tissues were extracted as follows: (1) Carcinoma of thyroid, woman aged 33 years, basal metabolic rate minus 6 before operation. (2) Quadriceps muscle from amputated leg. (3) Hemorrhagic fluid from aseptic necrosis of large nodule in adenomatous goitre, man aged 60 years, basal metabolic rate plus 21 five days before operation and plus 13 three days before operation. (4) A supposedly normal thyroid gland removed for cardiac disease, man aged 43, basal metabolic rate plus 7 before operation. (5) Pectoralis major muscle from radical mastectomy. (6) Toxic thyroid gland, woman aged 27 years, basal metabolic rate plus 24 just before operation, plus 43 a week previous.

The acetylcholine-equivalents of these extracts are given in Table I. Of the 5 extracts tested before and after atropinization, 2

TABLE I.

The acetylcholine-equivalent of six human tissue extracts. The use of the term acetylcholine equivalent does not imply that the substance tested is actually acetylcholine.

	Before atropine			After atropine		
	No. of animals used in assay		Aver. ac.-equiv.	No. of animals used in assay		Aver. ac.-equiv.
Extract	Dogs	Rabbits	γ per gm.	Dogs	Rabbits	γ per gm.
1. Carcinoma of thyroid	3	3	1.2	2	2	1.1
2. Quadriceps muscle	2	2	2.0	1	1	1.4
3. Thyroid cyst fluid	1	2	0.02	0	0	—
4. Normal thyroid	1	0	8.0	1	0	0.0
5. Pectoralis muscle	2	1	1.8	1	0	0.3
6. Toxic thyroid	2	2	3.6	1	1	5.6

showed only a slight change, 2 were less active and one more active after atropine. For the 2 that were less active after atropine, this conclusion was based on only a single assay in each case. It must be remembered, however, that after atropine, the blood pressure often falls somewhat and the absolute fall produced by an equivalent amount of substance may be less even though the relative fall is as great. The average acetylcholine-equivalent of the 2 normal human

[4] Wilson, W. C., Stewart, C. P., and Harkins, H. N., Depressor Substances in Burned Tissues, to be published.

muscle extracts was 1.9, that of the toxic thyroid was 3.6, the normal thyroid was 8.0, the carcinoma of the thyroid was 1.2, and the fluid from the thyroid cyst was 0.02. These results indicate that in these single instances, there is no unusual amount of depressor substance in toxic thyroid tissue or in carcinomatous tissue.

Controls. In addition to comparing the effects on atropinized and unatropinized dogs and rabbits with those produced by standard acetylcholine solutions, controls were made as follows: (a) 10% trichloracetic acid alone was found to produce a definite depressor action roughly 0.5γ ac.-equiv. per cc. acid. (b) 10% trichloracetic acid was extracted in exactly the same way as the human tissues. This included all steps of the process. Part of this solution was extracted with ether twice, part 4 times and part 6 times. All of these solutions were equally inactive, roughly less than 0.02γ ac.-equiv. per cc. acid. (c) Several of the tissue extracts were assayed without accurate neutralization after extraction and compared with the effects after neutralization to congo red solution and to phenolphthalein. In general these extracts were equally active. (d) Extract No. 5 (pectoralis major muscle) was assayed before and after being passed through a Mandler filter and found to be equally active in both instances.

There are many substances isolated from animal tissues that fall under the classification of depressor substances. These include (a) histamine, (b) acetylcholine, (c) choline and other choline esters, (d) adenosine, (e) P substance, (f) potassium, and (g) R substance.[1] The biological assay used in the present paper indicates that the extracts of human tissues tested contained practically no histamine and very little acetylcholine because they lowered the blood pressure of atropinized rabbits. The various other substances were not definitely excluded, but nothing was found to indicate that the active principle was other than the P substance. The amount of depressor substance in any of the human tissues tested was not as great as that found in certain animal tissues such as the spleen of the horse or ox which Chang and Gaddum found to contain 4 to 30γ ac.-equiv. This amount was said to be actually due to acetylcholine itself. These authors also found 28γ ac.-equiv. in extracts of a human placenta.

Conclusions. In several human tissue extracts, including toxic thyroid tissue and carcinomatous tissue, depressor substances were found not to be present in unusual amounts. The major part of the depressor substance present in these tissue extracts does not act like acetylcholine or histamine when tested for effect on the blood pressure of atropinized rabbits.