

Not infrequently when the capsular membrane and space are not demonstrable, the flagella may appear to originate from the somata. Since the capsular membrane may lie practically upon the bacterial bodies and since capsular membrane and soma stain the same color and, if close together, are indistinguishable, it seems not unlikely that in these cases the flagella, though appearing to arise from the bacterial bodies, arise in fact from the capsular membrane itself. The idea that flagella arise from capsule is further borne out by the fact that empty capsules (*i. e.*, devoid of somata) with flagella attached are frequently seen.

5. *Presence of capsule in the acid fast group.* We reported suggestive findings in the study of one member of the acid-fast group (*Mycobacterium tuberculosis bovis*). The observation was made on specimens stained overnight with carbol fuchsin, decolorized with acid alcohol and then stained by our capsule method. We have since obtained definite evidence of a capsule in this organism by exposing specimens for 3 or 4 minutes to the Casares Gil mordant, washing with water, staining with carbol fuchsin (heated for 3 or 4 minutes, allowed to stand for 10 minutes until cool). The organisms practically always lie in the capsule in an excentric position.

6186

A Quantitative Study of Adrenal Cortical Hormone Extraction.*

J. J. PFIFFNER, H. M. VARS, P. A. BOTT AND W. W. SWINGLE.

From the Biological Laboratory, Princeton University.

It was demonstrated^{1, 2} that cortical hormone can be prepared from whole beef adrenal glands by essentially the same methods of extraction used in the preparation of cortical hormone from dissected cortex.^{3, 4} The elaboration of a biological method of assay based on the minimum maintenance requirement of the adrenalectomized dog⁵ provided a reliable means of comparing the potency

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation of New York.

¹ Swingle, W. W., and Piffner, J. J., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 510.

² Swingle, W. W., and Piffner, J. J., *Am. J. Physiol.*, 1931, **98**, 144.

³ Piffner, J. J., and Swingle, W. W., *Anat. Rec.*, 1929, **44**, 225.

⁴ Swingle, W. W., and Piffner, J. J., *Am. J. Physiol.*, 1931, **96**, 153, 164, 180.

⁵ Harrop, G. A., Piffner, J. J., Weinstein, A., Swingle, W. W., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 449.

of whole adrenal extract with that prepared from dissected cortex. It has been shown that cortex extract (1 cc. represents 30 gm. beef adrenal cortex) contains 4 to 10 dog units (D. U.) per cc., the potency varying with different batches.⁵

Extracts prepared from whole glands are many times as potent as those prepared from dissected cortex (on an equivalent weight basis). Whole gland extract (1 cc. represents 40 gm. whole beef adrenal gland) contains 40 to 80 D. U. per cc. The following is a summarized comparison of whole gland and dissected cortex extract:

WHOLE GLAND	=	DISSECTED CORTEX
1000 gm.		720 gm. (average)
57.5 mg. active fraction		60 mg. active fraction (average)
25 cc. extract		24 cc. extract
40 to 80 D. U. per cc.		4 to 10 D. U. per cc.
Yield 1000 to 2000 D. U.		Yield 96 to 240 D. U.

Whole gland extracts of approximately equal potency but lower solid content can be prepared by using the same fractionation procedure as previously described but decreasing by 50% the thoroughness of extraction of the respective fractions. In this simplified technique the glands are extracted once with alcohol for 48 hours, the benzene soluble fraction is extracted twice with acetone, the acetone soluble fraction is distributed twice between 70% alcohol and petroleum ether and the alcohol soluble fraction is filtered only once through permutit. The adrenalin concentration is less than 1:2,000,000 (blood pressure-dog). Typical assay data follow:

TABLE I.
Assay on Whole Gland Extract (Half Method).
Ext. HM-2: 1 cc. = 40 gm. gland; 1 cc. = 2.0 mg. solids.
Dog No. 25.

Wgt. kilos	Daily Dose/kg.			Days	Urea N mg./100 cc.	Clinical Condition
cc.	mg. solids	gm. gland				
12.3	.2	.4	8	7	23-29	Normal
13.3	.1	.2	4	7	28-31	Normal
13.8	.05	.1	2	10	31-34	Normal
14.0	.025	.05	1	7	31-36	Normal
13.5	.0125	.025	0.5	7	31-36*	Variable appetite
12.9	.006	.012	0.24	5	35-90†	Definite insufficiency
Yield per kg. gland = 2000 D. U.						

* A dog unit is the minimum daily kg. dose of cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of 7 to 10 days under standard conditions previously described.⁵

† The end-point of the assay consists of a sharp rise in blood urea accompanied or very shortly followed by anorexia, loss in weight, decreased activity and in some cases spasticity of the hind-quarters.

Freezing the whole adrenal glands for 5 months prior to extraction has no effect upon the yield of hormone. A very good yield of hormone was obtained from glands which had autolyzed for 48 hours at room temperature. Extracts preserved with 0.1% benzoic acid at 5°C. retained their activity for 6 months. Longer periods have not been studied. The stability of the finished extract has been checked in two ways (1) adrenalectomized dogs have been kept on a single batch of extract for periods of 3 to 5 months. On withdrawing extract they have come promptly into insufficiency. (2) Assayed extract has been set aside for 6 months and re-assayed without showing any loss in potency.

6187

"Autosterilization" as a Problem in the Bacteriological Examination of Canned Foods.

F. W. TANNER, E. E. ECHELBERGER AND F. M. CLARK.

From the Department of Bacteriology, University of Illinois.

During investigations in which large numbers of cans of food spoiled by thermophilic bacteria were opened, many cans with evidences of spoilage contained no living bacteria even though examination of stained films revealed the presence of many cells. This seemed to indicate that the microorganisms responsible for the spoilage had probably died out. To secure more information on this situation which has become known as "autosterilization", the problem was attacked with canned corn and several spoilage bacteria including an active thermophilic spoilage organism, No. 1518, in both tin and large culture tubes.

Large culture tubes were half-filled with fresh canned corn, layered with paraffin and sterilized in the autoclave. The corn in the tubes was then inoculated with the spoilage organisms and divided into 4 sets. One set was incubated at 10°C.; the second at room temperature (20-37°C.); the third at 37.6°C., and the fourth at 55°C. At intervals of 2 or 3 days a tube from each group was removed, plated for the number of viable organisms, and the pH determined. A relationship between the temperatures at which the tubes were held and the number of living organisms was apparent with all cultures of thermophilic bacteria used. At 55°C. a lethal H ion concentration was reached, much more quickly than at 37°C.