

results were obtained in cases of toxic jaundice including catarrhal jaundice, and in obstructive jaundice of moderately long standing. In all these cases a definite diminution in the rate of hippuric acid excretion occurred, and significantly, the hourly output often remained low long after the jaundice had cleared up. In Table I a few typical results are recorded.

TABLE I.  
*Hourly Excretion of Hippuric Acid.*

Time hr.	Normal			Obstructive Jaundice		Catarrhal Jaundice	
	I gm.	II gm.	III gm.	gm.	gm.	gm.	gm.
1	1.29	0.43	0.81	0.54	0.43*	0.23	0.35*
2	1.96	1.96	1.46	0.93	0.56	0.51	0.51
3	0.82	1.28	1.51	0.93	0.54	0.65	0.44
4	0.28	0.79	1.49	0.81	0.70	0.54	0.69

\* 10 days after the first test.

## 6285

### Appearance of Large Amounts of Non-Stainable Cultivable Granules in Bacterial Cultures on Saccharose Media.

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A Gram-positive spore bearing aerobic bacillus, isolated following accidental contamination of a saccharose broth tube, was found to grow on a saccharose agar plate as a thick soft spreading film. This film later became of semi-liquid consistency and in it the bacteria could be seen whirling about. Using a dark background preparation, the culture consisted for the most part of scarcely perceptible minute granules, often arranged in large clumps. Masses of these granules were seen in saccharose broth cultures as well as upon solid media. Using a very strong light or sunlight with the dark field microscope, these granules were also visible in strong Brownian movement. They were not stained by the usual methods. They were made visible by the following technique: a loopful of a broth culture or of an emulsion of the agar growth was mixed on a slide with a loopful of a crystal violet solution (a 1:5 or 1:10 dilution of the solution used for Gram stain). About one minute later a loopful of a 5% solution of silver nucleinate was added and the whole mixture was spread over the slide as a thin film. A sec-

ond method also gave satisfactory results: the emulsion was spread over the slide, dried, stained with crystal violet without fixing, washed, covered with silver nucleinate without previously being dried, and finally the silver solution was allowed to drain off by tilting the slide. The bacteria appeared violet, the granules colorless on a brown background.

When a thick streak from a well grown culture is made on a saccharose agar plate and kept at 25°-30°C. the growth begins to spread in 15-30 minutes. With the aid of a hand lens the bacterial mass is seen to be surrounded by a thin film which spreads laterally at a rate of 2-3 mm. in an hour. Microscopically this film does not consist of bacteria, but of an amorphous material arranged in zig-zag branching streams and containing many tiny colony-like thickenings which are often connected with each other though in other areas connections between the tiny colony-like agglomerations cannot be made out. An impression preparation of this film reveals only the non-stainable granules without bacteria. As the film becomes thicker, the bacteria move actively into it from the edge of their colony, but this bacterial invasion is soon followed by a further extension of the spreading film. This phenomenon occurs only on saccharose plates, and not on plain, dextrose or blood agar.

The edge of this spreading film was touched with the tip of a fine glass capillary and another part of the plate inoculated. With considerable frequency clusters of tiny round colonies consisting solely of the non-stainable granules without admixture of bacteria developed. Definite growth of the transplanted colonies could be observed.

The filtrate of a thoroughly centrifuged saccharose broth culture through a Berkefeld N filter is usually turbid and on microscopic examination contains no bacteria but a large number of non-stainable granules. The filtrate remains sterile for many days and transplants of it on agar or in broth have not grown. However, in some filtrates contaminated in the course of repeated transfers the contamination was accompanied by an enormous increase in the number of granules, and on one occasion a large number of granule colonies grew out on a saccharose agar plate between the contaminating coccus colonies. When the spores of the bacteria were heated at 100°C. for one minute, and inoculated into saccharose broth the non-stainable granules were reproduced. It seems necessary to conclude therefore that they are derivatives of the bacterium

Oerskov<sup>1</sup> first described similar phenomena with a fluorescent-

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<sup>1</sup> Oerskov, T., *Zbl. f. Bakt.*, 1931, 1, 120.

like bacterium isolated from milk. We found this organism without difficulty in several fresh milk samples and were able to confirm with it most of the observations of Oerskov. We failed, however, with the milk organisms to demonstrate the most important point, that a growth of the non-stainable granules can be obtained which is certainly independent of the growth of the bacteria, though the evidence for this is strongly suggestive.

It has long been known that upon saccharose media the colonies of many bacteria develop a peculiar slimy consistency. We have examined the slime produced by 6 different types of organisms grown on saccharose media and have found it in all cases to consist of particulate matter in the form of non-stainable granules, similar in all respects to the findings described above. It is of special interest that streptococci from human throats often were found to form large semi-transparent colonies on saccharose plates. The granules in these colonies are very small and could be seen only with dark field illumination, being apparently too small to show up with the staining methods described above.

## 6286

**Colon Activation by Intravenous Hypertonic NaCl Injection in Unanesthetized, Trained Dogs.**

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Since Hughson and Scarff<sup>1</sup> first reported peristaltic augmentation of the ileum after NaCl intravenously in anesthetized cats, several investigators have confirmed the observation.<sup>2, 3, 4, 5</sup> Regarding the response of the colon no action has been experimentally demonstrated. Thus our results seem interesting since they show that the large intestine can be activated by salt solutions of adequate hypertonicity when intravenously administered.

This work was carried out in unanesthetized, trained collies,

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<sup>1</sup> Hughson, W., and Scarff, J. E., *Johns Hopkins Hosp. Bull.*, 1924, **35**, 197.

<sup>2</sup> Ross, J. W., *Canad. Med. Assn. J.*, 1926, **16**, 241.

<sup>3</sup> Dreyer, N. B., and Tsung, Thelma, *J. Pharmacol. and Exp. Therap.*, 1929, **36**, 629.

<sup>4</sup> Orr, T. G., Johnstone, P. N., and Haden, R. L., *Surg. Gynec. and Obstet.*, 1931, **52**, 941.

<sup>5</sup> Reid, P. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 220.