

amount of alkali as a stock solution of 1:1000, does not change the pH of the solution. The details of transmitting the heart beat to the chronograph are described in a previous paper.²

The hearts beat about 10 times in 20 seconds at first, gradually slowing to 10 beats in 30 seconds in 2 hours when the thyroxin, 1:10⁵, is added. No immediate change in rate occurs, the heart gradually slowing, with some rhythmic variation in rate, over a period of 2 days, when the rate may be 10 beats in 80 seconds. The 4 hearts studied showed no tendency whatever to increase in rate and behaved in every way like normal hearts, which likewise continually slow over long time periods. We, therefore, conclude that thyroxin has no delayed direct action on the heart after excision from the body. Its action in the body must be secondary, possibly the production of a substance which directly affects heart muscle and whose effect persists for some time. Epinephrin and ephedrin affect these hearts in the normal rate-increasing manner.

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Colony Variation in Pathogenic Strains of *Bacterium coli* Induced by the Use of Dyes.

CARL O. LATHROP.

From the Department of Bacteriology, Medical School, University of Buffalo.

There is perhaps no other single criterion for the identification of the culture type that is so significant as colony appearance. It is regrettable, however, that in many investigations slight or no effort is made to proceed beyond recording the colony appearance. That this procedure is ill-advised is evident to every investigator who finds discrepancies in the characteristics of apparently established types.

In connection with studies on the pathogenicity of certain strains of *Bacterium coli*, note was made of the fact that some dyes tended to cause the appearance of clearly defined rough colonies with marked consistency in cultures that had been quite uniformly smooth for many generations. Use was made of a series of dyes to ascertain whether this was a constant phenomenon, perhaps associated with specific dye radicals, and there were found 4 of 25 common dyes which gave definite results.

Methylene blue (1-1000) yielded 60% roughs on the first trans-

fer, with no satisfactory increase toward the desired 100% on repeated transfer. Crystal violet (Grübler, prewar stock) yielded 70-80% roughs on the first transfer with some tendency to increase on repeated transfer but falling short of 100%. Neutral acriflavine (1-1000) produced no roughs but practically 100% intermediates. Acriviolet, a mixture of crystal violet and neutral acriflavine, averaged about 95% roughs on the first inoculation, while the second, or at most the third transfer, even of thoroughly stabilized smooths, yielded 100% dissociation into rough colony types. The base used in these dye experiments was plain beef extract 3% agar, with no enrichment, hence there was no conflict between elements tending toward roughness and smoothness. A later report will deal with the restoration of roughs to smoothness, with a discussion of the effect of certain enriching substances favoring smoothness.

After the identity of the variants obtained had been established as true roughs with the single exception occasionally noted with other organisms, that motility was still active, the usual virulence tests were done and with the standard intraperitoneal inoculation the smooth strains maintained their original virulence and the rough strains proved avirulent. However, when intravenous inoculation was employed, the rough strains, 12 in number, all caused death of the inoculated guinea pigs in 24 hours or less. On autopsy, the lungs were markedly congested with a typical lobar pneumonia, and the organisms were recovered in each instance from the lungs. The autopsy cultures of the rough strains recovered from the lungs showed about 5% reversion to smoothness, which corroborated earlier observations that blood, while unable alone to accomplish reversion of roughs to smoothness, nevertheless favored such change.

The animals injected intravenously with the homologous smooth strains succumbed a few hours later with septicemia and no lung involvement. This was so unexpected and so subversive of the idea that avirulent strains had been obtained in the process of conversion from the normal smooth to the atypical rough that it was very carefully checked, using rabbits as well as guinea pigs. In every animal used, the rough strains, intravenously injected, caused acute death, usually in less than 20 hours, while the smooth ancestor strains consistently caused septicemia, from which cases autopsy cultures yielded undifferentiated smooth strains. Cultural studies of the rough variants showed them to be much more active fermenters of carbohydrates than their smooth ancestors. Further study is being made on the significance and mechanism of the described rough strain pneumonias.

I used 60 guinea pigs and 24 rabbits: (1) 12 guinea pigs, original virulence tests, intraperitoneal inoculation; (2) 12 rough variants from original smooth strains, intraperitoneal inoculation; (3) 12 same as (2), intravenous inoculation; (4) 12 same as (3), rechecks; (5) 12 smooth cross-checks from original smooth strains, intravenous inoculation; (6) 12 rabbits, smooth cross-checks, intravenous inoculation; (7) 12 rabbits, rough variants from original smooth strains, intravenous inoculation..

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Hyperplasia of Thyroid and Exophthalmos from Treatment with Anterior Pituitary in Young Duck.*

JOSEPH A. SCHOCKAERT.† (Introduced by P. E. Smith.)

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

It has been definitely shown that the normal structure of the thyroid gland is dependent upon an autacoid present in the anterior lobe of the pituitary gland. In amphibians its release at the time of metamorphosis stimulates the thyroid to excrete its content and induces the increased cellular activity characteristic of this period. In mammals Loeb and his coworkers^{1, 2} and Aron^{3, 4} showed that the injection of anterior pituitary extracts and emulsions produced also some characteristic changes in the thyroid suggestive of a higher activity. The follicular epithelium became higher, mitoses were present and colloid was excreted. The glands increased in size from 2 to 4 times and became more vascular. The metabolism of treated guinea pigs, as shown by Siebert and Smith⁵ was markedly increased. As previous observations⁶ had shown that young ducks were very sensitive to pituitary extracts and emulsions, it seemed

* Aided by a grant from the Special Research Fund of Columbia University to the Department of Anatomy.

† Fellow of the C.R.B. Educational Foundation.

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³ Aron, M., *C. R. Soc. Biol.*, 1929, **102**, 682.

⁴ Aron, M., *Revue fr. Endocrin.*, 1930, **8**, 472.

⁵ Siebert, W. J., and Smith, R. S., *Am. J. Physiol.*, 1930, **95**, 396.

⁶ Schockaert, J., *Arch. intern. Pharm. Thérap.*, 1931, **41**, 23.