minute, the average being for two such extended periods 15 and 22 seconds, respectively. During the periods of rapid ejection the tract of the worm remains more or less distended with blood. One gains the impression that the worm gorges itself with blood before starting to eject. The red material which gradually fills the intestine may be readily seen through the transparent tissues of the worm. The anal end becomes dilated and immediately there occurs a spasmodic contraction, often of sufficient force to move the whole posterior part of the worm. A droplet appears with great suddenness from the anal orifice. It was noticed in the case of some worms that blood may finally cease to replace that ejected and that the worm may gradually become almost white or colorless. When a worm was seen to move to a new point of attachment or when it disengaged itself from a part of the mucosa to which the blood supply had been cut off it was usually white, with little or no blood visible in its tract. Some worms were observed which never became colorless during the entire day. But in these as in the others there were periods when no blood was emitted. In the case of one dog the worms observed were relatively inactive. Periods during which little or no blood was ejected lasted for half an hour or more, and both the frequency and the size of droplets during the active periods tended to be less than in the first experiments. But even in this instance there were occasionally short periods during which large droplets were emitted at intervals of less than a minute.

The color of the blood emitted was sometimes purplish like venous blood, sometimes bright red or arterial in hue. The color seemed to be a characteristic peculiar to the individual worm and hence probably dependent on the local nature of the blood supply at the point of attachment. There was no distinct evidence of any change of color, from red to blue, as the blood passed through the worm. This point may need further study, for one is impressed with the possibility that the enormous amount of blood ingested by the parasite may serve a respiratory function.

The size of the ejected droplet was estimated by collecting a single emission in a capillary pipette containing isotonic salt solution. The blood was mixed and made to a definite volume of 0.5 or 1.0 cc. The red corpuscles were counted in the usual manner. Calculation gives the total number of red cells emitted per drop. In the case of two drops, each from a different worm, there were 1,168,000 and 1,268,000, respectively. Assuming the erythrocyte count of the dog to have been 5,000,000 per cu mm, the droplets would therefore each represent the red cells from approximately 0.25 cu mm of the dog's blood. Knowing the size of the droplet one could easily estimate the daily loss of blood incurred at the expense of the host provided one knew the average rate of ejection by each worm and the number of worms present. Although the normal average rate of ejection is not known at present, one can gain an idea of the possibilities by assuming a rate of, let us say, one ejection per minute per worm, which is certainly not an excessive maximum, as judged by two experiments lasting for over seven hours. On this assumption there will be removed from a dog in this manner 1 cu mm in 4 minutes, 15 cu mm per hour or 360 cu mm per day by a single worm. With 1,000 worms present and similarly active the loss to the host would be 360 cc per day. In this calculation no allowance is made for blood cells digested by the worm, nor, of course, for blood that may be lost by direct hemorrhage at the point of attachment to the mucosa.

Although at present one can only speculate as to the actual amount of blood discharged by the worms under normal conditions, there is every reason to believe that the parasites not only can but do take in and expel much more blood than could be accounted for by their food requirements alone. As to what biological purpose is served by this process, so wasteful of the blood of the host, it is impossible at present to judge.

Although these observations do not tell us anything of the behavior of those forms of the parasite which infest man, they do suggest that the factor of blood-sucking should be reconsidered, on the possibility that this activity of the worms may be found to play a part more important than formerly suspected in the production of anemia in human cases.

HERBERT S. WELLS
DEPARTMENT OF PHARMACOLOGY,
VANDERBILT UNIVERSITY MEDICAL SCHOOL

A RELATION BETWEEN ROTENONE, DEGUELIN AND TEPHROSIN

IN a previous communication to this journal[1] it was stated that apparently the principal toxic constituents of derris and cubé roots, namely, rotenone, toxicarol, deguelin and tephrosin, were, from a chemical standpoint, more or less closely related. At the time the report referred to was made, only indirect evidence supporting the assumption was available. This consisted of the similar solubilities of the compounds in many solvents, the identity or close similarity of their empirical formulas, the fact that all contained two methoxyl groups, and, finally, their general behavior toward certain reagents.

As the study of the chemistry of these materials has

[1] E. P. Clark, SCIENCE, 71: 396, April 11, 1930.

progressed, direct evidence of a relationship among three of the compounds has been shown. The essential facts are as follows. Rotenone upon mild oxidation yields dehydrorotenone $C_{23}H_{20}O_6$. This compound when boiled with alcoholic potassium hydroxide and zinc dust gives rise to a hydroxy acid[2] $C_{23}H_{24}O_8$, which, when oxidized with hydrogen peroxide, yields derric acid[3] $C_{12}H_{14}O_7$. Derric acid contains the two methoxyl groups originally present in rotenone and represents one half of the rotenone molecule.

Upon oxidation with potassium ferricyanide deguelin $C_{23}H_{22}O_6$, the light green compound melting at 171° which is found in derris and cubé roots, the leaves of *Cracca vogelii* and the roots of *Cracca toxicara*, gives dehydrodeguelin $C_{23}H_{20}O_6$. This substance, analogous to dehydrorotenone, yields on boiling with alcoholic potassium hydroxide a phenolic monocarboxylic acid $C_{23}H_{24}O_8$, which has been called deguelic acid. Deguelic acid when oxidized with hydrogen peroxide in the same manner as was the acid from dehydrorotenone also yields derric acid.

Tephrosin $C_{23}H_{22}O_7$ when treated with a mixture of sulphuric and acetic acids or with acetic anhydride loses the elements of water and forms dehydrodeguelin. Thus derric acid constitutes one half of the molecule of rotenone, of deguelin and of tephrosin. The evidence also shows that tephrosin is intimately related to deguelin, since the loss of one molecule of water from tephrosin gives dehydrodeguelin. Without further experimental evidence, it appears probable that tephrosin is a hydroxydeguelin. Detailed reports of this work will appear elsewhere.

E. P. CLARK

INSECTICIDE DIVISION,
BUREAU OF CHEMISTRY AND SOILS

DISSOCIATION OF BACTERIUM GRANULOSIS NOGUCHI AND IDENTIFICATION OF THE ORGANISM BY MEANS OF RABBIT IMMUNE SERA

THE viability of *Bacterium granulosis* for periods of a year or more on the semisolid ("leptospira") medium of Noguchi has already been recorded.[1] Recently, on transfer to blood agar of a culture which had stood for 8 months on semisolid medium without transfer, and which had shrunk by evaporation from 8 cc to 2 cc or less, a growth was obtained of discrete, yellowish gray, opaque, dry, bead-like colonies, with rough surface, which were distinct from the semi-transparent, grayish, mucoid, confluent colonies usually seen in young cultures of *B. granulosis*. Microscopic examination, however, showed a morphology typical of *B. granulosis*. The strain fermented the usual carbohydrates, and agglutination tests with immune sera prepared in rabbits by means of the ordinary type cultures yielded clearly positive results.

A few smooth mucoid colonies were found among the rough dry ones, and a pure smooth strain was readily isolated from one of these. Plating of rough colonies yielded a growth chiefly of the rough type, with gradual reversion to smooth within 3 or 4 days in those portions of the plate where the colonies were widely separated. Replating every 24 to 48 hours reduced the tendency to reversion until it has practically disappeared. The tendency to the formation of yellow pigment, which is ordinarily seen only in old cultures of *B. granulosis*, is much enhanced in the rough cultures and appears early. The dissociation has since been found in other strains of *B. granulosis*, the identity of which had been uncertain until they were found to be agglutinated by immune serum.

Agglutination tests have so far proved the most useful means of identifying unknown cultures, since fermentation tests may vary occasionally from strain to strain. The serum[2] is highly specific for *B. granulosis*. Fourteen known strains of the organism so far tested have been agglutinated in dilutions of 1:256 to 1:1024, while no agglutination takes place in the case of the common bacteria found in the conjunctival secretions or tissue of man or monkey (*M. albus, M. aureus, B. xerosis, B. influenzae*), or of numerous gram-negative bacteria from the same source.

E. B. TILDEN

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK

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[2] A. Butenandt, *Ann. d. Chem.*, 464: 272, 1928.

[3] F. B. LaForge and L. E. Smith, *Journal Am. Chem. Soc.*, 52: 1091, 1930.

[1] Tilden, E. B., and Tyler, J. R., *J. Exper. Med.*, 1930, 52, 617.

[2] The sera have been prepared by injecting rabbits intravenously at 5- to 6-day intervals with gradually increasing doses (1 cc to 5 cc) of heavy suspensions of *B. granulosis* grown on freshly prepared nutrient agar in Blake bottles.