

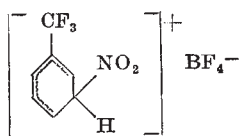
acylating reactions (for example, in this case in the nitration) as the fatty acid fluorides. In earlier work it was possible to use the nitryl fluoride-boron trifluoride system as the isolated stable nitronium borofluoride complex to work out a new nitration reaction².

As a consequence of the great reactivity of nitryl fluoride, in this case the preparative difficulties are much greater than in the acylating reactions of the fatty acid fluorides catalysed by boron trifluoride³. Moreover, the nitryl fluoride is also a strong oxidizing agent and even a fluorinating agent. The use of methylbenzenes is not to be recommended in the nitrating reaction catalysed by boron trifluoride because of the reaction velocity of the activated aromatic nucleus, which is so great that the reaction takes place even at -100° with explosion.

We have found that benzotrifluoride is a suitable model for our experiments. The melting point is relatively low, -29° , the CF_3 group shows a strong $-I$ effect and de-activates the nucleus, and in consequence the reaction with nitryl fluoride is moderated; at temperatures less than -20° no reaction is observed. Nitronium borofluoride itself nitrates benzotrifluoride only at $+100^\circ$.

We have prepared the nitryl fluoride needed for our experiments according to the method of Schmeisser and Elischer⁴ with the thermal decomposition of nitronium borofluoride with sodium fluoride.

We added 14.5 gm. (0.1 mole) benzotrifluoride carefully in small portions to 6.5 gm. (0.1 mole) nitryl fluoride at -80° . No reaction was observed; then we added 0.2 mole boron trifluoride to the system at -120° . After homogenizing, the reaction mixture was allowed to warm slowly up to -100° , during which the excess of boron trifluoride distilled off. 0.1 mole boron trifluoride was held back in the reaction mixture, showing that a 1 : 1 : 1 nitryl fluoride : benzotrifluoride : boron trifluoride complex was formed. The yellow solid complex was stable up to -50° , when it decomposed without melting with strong evolution of boron trifluoride and with a nearly quantitative yield of *m*-nitro benzotrifluoride. Although in this case the complex has no sharp decomposition point and the specific conductivity could also not be determined, still we think that, according to the analogy of the earlier work, in which the stable intermediate complexes of aromatic substitutions were investigated¹, in this case also the 1 : 1 : 1 molecular complex can be formulated as an onium salt (or σ -complex) with the following structure :



G. OLÁH
L. NOSZKÓ
A. PAVLÁTH

Chemical Central Research Institute,
Hungarian Academy of Sciences,
Hungária-krt. 114,
Budapest XIV.

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Blood Group P Substance in Hydatid Cyst Fluids

In the past eight years, 132 cases of hydatid disease have been treated in the Auckland Group of Hospitals. Among these patients, two were found to have strong anti-P agglutinins in their sera. These were recognized in the course of cross-matching blood for transfusion. About ninety of these cases had blood cross-matched, and of these only 25 per cent could be expected to be P-negative and able to have anti-P agglutinins in their sera. It seemed that this was a selected group presenting a high proportion of unusually strong anti-P sera. Merritt and Hardy¹ reported a serum containing strong anti-P in a boy aged twelve years with hydatid disease.

The association of hydatid infestation with strong anti-P led us to examine some hydatid cyst fluids for anti-P inhibiting substances which may have been responsible for the stimulation of anti-P agglutinins in these cases. The method used was that described by Race and Sanger² for testing for A and B substances in saliva, but substituting a strong anti-P serum and strongly reacting P-positive red cells for the anti-A and anti-B sera and A and B cells.

The dilutions of the cyst fluids in saline were carried to at least 1 in 128. The hydatid cyst fluids were obtained from cysts in the livers of sheep, and in each cyst the presence or absence of live scolices was noted. The results are shown in Table 1.

It will be seen that there is no inhibition of anti-P where scolices are absent, and considerable variation in the concentration of the inhibiting substances where live scolices are present. It is probable that the presence of specific inhibiting substances is dependent upon an active germinal layer of the cyst wall. No diminution of anti-P inhibition was observed after boiling the cyst fluids in a water-bath for 10 min., centrifuging, and using the clear supernatant fluid in the tests. Further, it was found that no inhibition of the specific activity of the various other blood-group antisera available resulted when these were incubated with an equal volume of undiluted fluid obtained by pooling the fluids of nine cysts. The antisera tested were anti-A, -B, -M, -N, -S, -Le^a, -H, -Lu^a, -D, -C, -C^w, -c, -E, -Kell, -Fy^a. The temperature used for the absorption and the

Table 1

Cyst fluid No.	Maximum dilution of hydatid fluid showing complete inhibition of anti-P	Absence, presence and condition of hydatid scolices
1	32	Active scolices
2	64	Active scolices
3	No inhibition	No scolices
4	128	Active scolices
5	64	Active scolices
6	32	Active scolices
7	256	Active scolices
8	64	Active scolices
9	32	Active scolices
10	No inhibition	No scolices
11	No inhibition	No scolices
12	4	Few degenerate scolices
13	No inhibition	Few degenerate scolices
14	32	Active scolices
15	16	Few active scolices
16	No inhibition	Degenerate scolices
17	No inhibition	Degenerate scolices
18	64	Active scolices
19	32	Active scolices
20	128	Active scolices
21	128	Active scolices
22	128	Active scolices
23	128	Active scolices
24	128	Active scolices
25	128	Active scolices

method for testing the absorbed serum with appropriate red cells were those suitable to the individual antiserum.

The possibility of producing potent anti-P testing sera by immunizing rabbits with selected hydatid cyst fluids is being investigated.

G. L. CAMERON
J. M. STAVELEY

Blood Bank,
Central Laboratory,
Auckland Hospital,
Auckland,
New Zealand.
Aug. 7.

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Hæmoglobin in the Crustacea

HÆMOGLOBIN in solution in the blood plasma is found in numerous entomostracan Crustacea. Ray Lankester first discovered it in *Chirocephalus*, observing the absorption bands of oxyhæmoglobin with a microspectroscope¹, and soon afterwards found it in *Daphnia*. The wave-length of the α -band differs among species within the genus *Daphnia*². The pigment also occurs in the blood of other Cladocera, for example: *Sida*, *Simocephalus*, *Moina*, *Bosmina*, *Ilyocypris*, *Eurycercus*, *Leydigia*, *Chydorus*, etc., but not in *Leptodora*. The hæmoglobin of *Ceriodaphnia* has a higher oxygen affinity than that of *Daphnia*, and the former lives in fouler water³. Hæmoglobin is present, too, in Phyllopoda: it is in the blood of Conchostraca (*Lyceus*, *Leptestheria*⁴, *Limnadia*), Anostraca (*Chirocephalus*, *Artemia*^{5,6}) and Notostraca (*Triops*⁷, *Lepidurus*). It may be universal in the blood of the Phyllopoda. Among the Ostracoda, hæmoglobin occurs in *Cypria*⁸ and *Pseudocypria*.

In the Copepoda, the parasites *Lernaeocera*⁹ and *Mytilicola*¹⁰ have hæmoglobin in the blood, but hitherto the pigment has been unknown in free-living forms. I have not detected it in *Diaptomus* or *Cyclops*; but lately have found it in the blood of various species of Harpacticoida. The blood of these animals does not circulate, as there is no heart. Hæmoglobin was first detected in a new marine species, *Laophonte foxi* Harding¹¹, living in mud; but none could be found in the common species *Tisbe furcata* (Baird), moving freely in sea water. Hæmoglobin is present in the blood of the common freshwater species *Canthocamptus staphylinus* Jurine, found on and in mud, but it is absent from *Bryocamptus pygmaeus* (Sars), on wet moss. *Attheyella crassa* (Sars) from the bottom of the Lago Maggiore at a depth of 120 m. and from moss in a rivulet at Pallanza in Italy had hæmoglobin, but the concentration was considerably greater in the former situation. A full account of this investigation will be published elsewhere.

Among the Cirripedia, hæmoglobin is unknown in the barnacles, although it is present in the blood of some of the parasitic Rhizocephala: among these there is none in *Sacculina*, but it occurs in *Septosaccus*¹² and *Pellogaster*¹³. Not only is hæmoglobin thus found in the blood of numerous Crustacea, but it also occurs in a variety of tissues in *Daphnia*, namely, muscle, nervous system, fat cells¹⁴ and eggs. This is in contrast to the Vertebrata, where the pigment is only present in red blood cells and in muscle. In the Cladocera and Phyllopoda the concentration of

hæmoglobin in blood and tissues varies inversely as the dissolved oxygen concentration in the water, the changes being both rapid and considerable when animals pass from one water to another¹⁵.

Hæmoglobin, we have seen, is present in Branchiopoda, Ostracoda, Copepoda and Cirripodia, yet it is not known to occur in the higher Crustacea, the Malacostraca. In two malacostracan groups, the Decapoda and Stomatopoda¹⁶, the copper-containing respiratory protein hæmocyanin, blue in the oxidized state, occurs dissolved in the blood plasma. Hæmoglobin has indeed been reported in a species of the Amphipoda, *Urothoe grimaldii* Chevreux¹⁷, which burrows in sand on the sea-shore. I have, however, been unable to confirm this report, finding no hæmoglobin in animals of this species from near Plymouth: 30 individuals were examined spectroscopically on three different occasions.

H. MUNRO FOX

Queen Mary College,
University of London.
Dec. 10.

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Presence of a Substance Rich in Protein-bound Cystine or Cysteine in the Neurosecretory System of an Insect

COMPARISONS have been drawn between the hypothalamo-hypophysial neurosecretory system of vertebrates and corresponding systems in invertebrates; for example, the pars intercerebralis-corpora cardiaca neurosecretory system of insects¹. Such comparisons are supported by the demonstration in both groups² of a deeply staining chrome-alum-hæmatoxyphil neurosecretory material. In the dog, this material has been described as a glycolipoprotein 'bearer-substance', soluble in lipid-solvents, and for this reason clearly to be differentiated from the posterior pituitary principles³. There is, however, an alternative view⁴, namely, that chrome-alum-hæmatoxyphil vertebrate neurosecretory material, or material in its exact distribution, is essentially a protein, which, in tissues which have not been fixed in formalin, is soluble in water rather than lipid solvents, and which for these reasons, and because of its high cystine content, could well be closely akin to the posterior pituitary principles. The present investigation is concerned with the nature of neurosecretory material in the intercerebralis-cardiacum system of the cockroach, *Leucophaea maderae*.

Prof. Berta Scharrer very kindly provided me with tissues from five adult cockroaches, fixed in Helly's fluid (formol-Zenker), embedded in paraffin wax, and serially sectioned. Representative sections, stained