

filters so large as in those species; the filaments are arborescent, as in other microhylids, and not as in *Rana* or *Bufo*.

The animals are clearly highly specialized for microphagy, since the oesophagus, already small in comparison with that of *Rana* or *Bufo*, has its lumen still further reduced by the thick septum, so that only narrow mucous cords can enter. They appear well adapted for life in a normal pond, having neither the huge filters (with rudimentary gills) needed for life in oligotrophic waters, nor the well-developed gills (with small filters) needed for life in a puddle. They were found in such a pond.

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¹ Noble, G. K., *Bull. Amer. Mus. Nat. Hist.*, 58, Art. 7, 291 (1929).

² Savage, R. M., *Proc. Zool. Soc.*, 122 Part 2, 467 (1952).

Hæmolytic Disease of New-born Pigs caused by Iso-immunization of Pregnancy

HÆMOLYTIC disease of the new-born is now well recognized in the human subject and is known to be due to iso-immunization of the mother by foetal red cell antigens of the *Rh* group. Within the past few years the condition has been observed in foals^{1,2}; but so far as we are aware, its existence as a naturally occurring disease has not yet been established in any other species, although the possibility of a comparable pathogenesis in other animals has been suggested.

The classic pattern of hæmolytic disease consists in the mating of serologically incompatible parents. As a result the foetus inherits antigen from the father which is passed to the mother and against which she produces antibody. This antibody, which has power to destroy the red blood cells of the young animal, may, in favourable circumstances, pass back into the foetus or it may become concentrated in the mother's milk. The clinical condition of icterus gravis neonatorum has long been appreciated in human as well as in veterinary medicine; but more recently it has come to be recognized as a common symptom of hæmolytic disease.

Recently, we have carried out investigations which establish the existence of hæmolytic disease in pigs, following serious losses among baby pigs within the first week of life on one large farm in the Midlands³. In a high proportion of cases there were no survivors from affected litters; although all piglets appeared to be healthy at birth, varying degrees of clinical jaundice became evident at about the forty-eighth hour, and it was usual for the entire litter to be dead by the fifth day. At autopsy, icterus of the skin, musculature and viscera were constant lesions, and it was common also to find congestion and enlargement of spleens, some of which were nearly black in colour. Perhaps the most striking feature at post-mortem examination, however, was provided by the quantity of blood-stained peritoneal fluid which was present in nearly all carcasses, and this, together with the darkened urine, served to indicate the speed at which red blood cells were being broken down by maternal iso-antibody. In general, post-mortem findings provided evidence of acute disease and depicted rapidly progressing hæmolysis.

With the generous assistance of Dr. R. R. A. Coombs, of the Department of Pathology, Cambridge, it was possible to apply the serological techniques of

Rhesus factor work to the diagnosis of the disease in pigs and to demonstrate the existence of iso-immunization. Citrated blood samples from affected piglets were subjected to the direct antiglobulin sensitization test (Coombs test), using anti-pig globulin serum prepared in rabbits. Rapid and complete agglutination was obtained of all samples. Parental blood incompatibility was shown also by well-marked reaction between serum from affected sows and the red cells of the boar.

Although in hæmolytic disease of the human infant due to iso-immunization, icterus gravis neonatorum is common, it is by no means constant, and the condition may provoke a wide variety of symptoms. Very little knowledge exists yet concerning the manifestations of the disease in animals; but it seems reasonable to assume that they may be as varied as those of man and that some well-known clinical entities, the cause of which is still obscure, may be shown to be associated with maternal iso-immunization. Much work needs to be carried out into this kind of disease in animals, for with the necessary information relating to blood groups of the various species might emerge knowledge which could lead to the avoidance of losses resulting from the mating of serologically incompatible parents.

Note added in proof. Since this article was written we have learned of the work of Szent and Szobó into hæmolytic jaundice of new-born pigs in Hungary⁴.

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¹ Carroll, H., and Bessis, M., *C.R. Acad. Sci., Paris*, 224, 969 (1947).

² Coombs, R. R. A., Crowhurst, R. C., Day, F. T., Heard, D. H., Hinde, I. T., Hoogstraten, J. L., and Parry, H. B., *J. Hyg.*, 46, 403 (1947).

³ Buxton, J. C., and Brooksbank, N. H., *Vet. Rec.*, 65, 287 (1953).

⁴ Szent, I. T., and Szobó, I., *Mag. Allator. Lap.*, 7, 331 (1952).

Paper Chromatography of Keto-Acids

BLOOD pyruvic acid estimations were being made in this laboratory, and it was important to test the specificity of the method employed. The acid was extracted as the hydrazone by the method of Friedemann and Haugen¹ (using toluene for the extraction) and the hydrazones chromatographed under the conditions which Altmann *et al.*² had found successful.

Human blood was deproteinized with 10 per cent metaphosphoric acid and analysed for pyruvic acid¹; the final ice-cold sodium carbonate solutions of the phenylhydrazones were acidified with concentrated hydrochloric acid and extracted into ether, which was removed under reduced pressure.

The hydrazones from blood were run with the hydrazones of pyruvic acid, phenyl-pyruvic acid, α -ketoglutaric acid, acetoacetic acid and acetone, which were prepared by incubating the keto-compound with a 0.1 per cent solution of 2:4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The precipitated hydrazones were centrifuged, and the precipitates washed twice with 2 *N* hydrochloric acid and twice with water.

The chromatograms were photographed through a Wratten tricolour blue filter. The accompanying photograph illustrates the main findings. The average