the mice not injected with P^{32} died after several weeks on the low protein diet with added sulfasuxidine. On the other hand, in the groups of mice injected with 5 μc/g of P^{32}, the % of survivors at the 21st day and the time of 50% deaths were the same as, or greater than the values previously observed in mice injected with a smaller dose of P^{32} (4 μc/g) and maintained on a diet without added sulfasuxidine (Diet 31, which differed from Diet 34 only in a higher fat content) (1). It seems therefore that the inclusion of sulfasuxidine in the diet did not enhance the susceptibility of the animals to the injurious action of the isotope.† Supplementation of the sulfasuxidine-containing diet with folic acid alone increased the % of survivors at the 56th day, but the increase was not statistically significant ($P>0.05$). Vit B_{12} seemed more effective, however only the increase in the % of survivors was significant. On the other hand, the combined administration of folic acid and vit B_{12} offered a marked protection with a high degree of significance for both % of survivors and survival time. The further addition of menadione and biotin did not enhance the beneficial action of the mixture of folic acid and vit B_{12}. Altogether our results suggest that none of the vitamins tested modify appreciably the damage caused by the introduction of P^{32}, but that an adequate supply of both vit B_{12} and folic acid may be an important factor for the recovery of the damaged tissues.

The hematological changes following the introduction of P^{32} in mice on the sulfasuxidine-containing diet showed approximately the same general behavior in all groups of Series IV. After an initial fall, the values rose progressively and often declined again at the longest intervals. However, between the 12th and the 36th day, that is, during the recovery period after the initial fall, the % hemoglobin and the red and white cell counts in the blood of mice on the diet supplemented with both folic acid and vit B_{12} were all higher than in the other groups. Because of the limited number of determinations, the differences observed can only be taken as a suggestive indication for a relationship between the improved hematological picture and the prolonged survival of mice receiving the mixture of the two vitamins.

Summary. The addition of generous amounts of folic acid, or folic acid and vit B_{12}, to experimental diets had no significant effect on the survival of mice injected with a dose of P^{32} in the higher range of the LD_{50} (21st day). On the other hand, when sulfasuxidine was added to the diet and the mice were injected with a dose of P^{32} slightly below the LD_{50} (21st day), the administration of vit B_{12} and folic acid increased significantly the time of 50% deaths, the average time of survival and the % of survivors (at both the 21st and the 56th day).

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† Several recent reports suggest that antibiotics may even be beneficial in the treatment of radiation injury by preventing general or localized infections. For example, streptomycin and penicillin effectively reduced the mortality or prolonged the survival of rats injected with P^{32}(11).

11. Koletsky, S., and Christie, J. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 363.

Development and Secretion of the Blood Group Factor O in the Newborn. (18554)

TIZIANO G. FORMAGGIO. (Introduced by A. S. Wiener)
From the Institute of Legal Medicine of the University of Pavia.

As part of a series of investigations on the development and secretion of blood group properties, we have investigated the development and secretion of the O property in the newborn, child, and adult. The development of the O property has been studied by determining the agglutinability and group-specific absorbing power of red blood cells, using

an anti-*Shigella dysenteriae* immune goat serum. This serum was absorbed by mixing one volume of the serum with three volumes of saline and one volume of packed washed A_1B cells; the mixture was allowed to stand in the refrigerator for 30 minutes, and the serum was separated by centrifugation. The absorbed serum gave strong positive reactions with all O and A_2 cells, and only weak reactions with a certain percentage of bloods of group A_1 and B, in conformity with Hirszfeld's theory. The same serum in a 1:8 dilution reacts exclusively with group O and group A_2 blood cells. These observations were made in tests on a series of approximately 4,000 blood specimens.

The absorbing power was determined by incubating for one hour at 37°C the serum diluted 1:4 in saline solution with one-fourth volume of washed, packed red cells; the absorbed serum was titrated against standard group O test cells from an adult. As a control, parallel absorption experiments were carried out with a standard specimen of group O red blood cells. In all the experiments the red blood cells of the same group O adult, with which the anti-O serum was titrated, were always used as controls. The blood to be tested was almost always obtained by venipuncture; except in young children when it was obtained at times from the heel. The tests were carried out on 75 newborn infants (from the Obstetrical and Gynecological Clinic of the University of Pavia) on their first and second days of life, and repeated after a fortnight. Blood samples were also collected from 100 children (Pediatrics Clinic of the University of Pavia) of ages ranging from a few days to 7 years all of whom were either healthy or had no blood disease. In addition, blood samples were collected from 50 individuals of ages 10 to 30 years, from students, blood donors, and other healthy persons. The tests on the children and adults were repeated 3 to 5 times at intervals of at least one week. In the younger children (one to 12 months of age) the tests were also made at intervals of 6 months. In this way it was

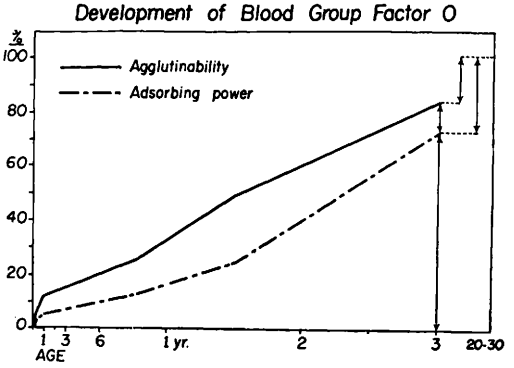

FIG. 1.

possible to demonstrate the development of the O agglutinogen in the same individual as well as in a random group of newborn and adults. The O substance in the saliva was determined with the same anti-*Shiga* immune goat adsorbed serum, diluted with 32 volumes of saline solution (*i.e.*, at 1:8 titer) by a quantitative method(1) and using progressively doubled dilutions of the saliva, collected from newborn infants and very young children with a thick cotton swab rubbed over the floor of the mouth.

The results were fairly uniform for each of the different ages and may be summarized as follows (Fig. 1): the agglutinability of group O blood cells of newborn infants and of infants during the first days of life, determined by an anti-O serum (goat anti-*Shiga* immune serum absorbed with A_1B blood cells) shows values which may vary from 1:4 to 1:16 (control tests with adult O blood cells gave titers up to 1:256); only exceptionally were titers against newborn cells higher. The agglutinability increases progressively with age, and approaches adult values after one to 2 years; at the age of 5 years values identical with those of adults were observed.

The group-specific absorbing power has been found to be considerably lower than in adults, and correlated with the agglutinability. The absorbing power also increases progressively with age, in parallel with the agglutinability. These results are in conformity with

1. Formaggio, T. G., *Min. Medicoleg.*, 1950, v70, 103.

2. Formaggio, T. G., *Atti 3rd Congr. Internaz. Transf. Sangue*, Torino, 1948, v1, 185.

those found(2) when investigating the development of the agglutinogens A and B in the newborn and infant.

The amount of O substance in saliva as determined by its capacity to neutralize the anti-O serum has been found approximately equal to that of saliva of adults of the "secretor" type; hence, it may be concluded that there is no direct proportional relation between the quantity of antigen present in the red blood cells and the quantity of antigen present in the body fluids. The relative frequency of the secretor and non-secretor types is the same in newborn, in children, and in adults of O group.

If one may assume without qualification that our serum reveals the existence of a special antigen peculiar to the blood group O, our results could be interpreted in the same way with those obtained with the agglutinogens A and B [Kemp(3), Morville(4), Thomsen and Kettel(5), Formaggio(2)]. Recent reports by Boorman, Dodd and Gilbey(6), by Morton and Watkins(7), by Grubb(8) and others, on the nature of the O substance and on the heterogenetic antigen "H," however, suggest that our experimental results should perhaps be interpreted more cautiously.

From our own experimental data it seems that the supposed essential difference between O and H antigens cannot be considered as proved; moreover, it cannot be denied that animal anti-O sera (and in particular the goat anti-*Shiga* immune serum) show group O specificity, and not merely a generic H specificity. It may be presumed that anti-O sera do not contain a single specific antibody, but contain a complex mixture of antibodies of different specificity, the proportions of which vary in sera of different origin, and this may account for the different results obtained by various workers. Among these antibodies there is undoubtedly at least one which is truly O specific (perhaps related to the product of Bernstein's gene *O*). It is probably with such anti-O sera that the behavior of agglutinogen O of red blood cells, analogous to that of agglutinogens A and B, is best demonstrated.

Summary. The development of the property O in the newborn and in young children, investigated from the point of view of the agglutinability and group-specific absorbing power with an immune anti-*Shigella dysenteriae* goat serum, is comparable to that of the agglutinogens A and B, namely, the agglutinability and the absorbing power are both extremely poor at birth, but increase until after 3 to 5 years their values become equal to those of the adult. Saliva from newborn and children of group O of the "secretor" type, neutralizes anti-O serum approximately to the same degree as saliva of adults of group O. The results obtained seem to contradict recent investigations which consider the so-called anti-O sera to have only an anti-H specificity. Probably, anti-O sera contain a mixture of serologically related antibodies to some of which cannot be denied an anti-O specificity *sensu strictiori*.

3. Kemp, T., *C. R. Soc. Biol.*, 1928, v99, 417.

4. Kemp, T., *Acta path. microbiol. scand.*, 1930, v7, 146.

5. Thomsen, O., and Kettel, K., *Z. Immunitäts.*, 1929, v63, 67.

6. Boorman, K. E., Dodd, B. E., and Gilbey, B. E., *Ann. Eugen.*, 1948, v14, 201.

7. Morgan, W. T. J., and Watkins, W. M., *Brit. J. Exp. Path.*, 1948, v29, 159.

8. Grubb, R., *Acta path. microbiol. scand.*, Suppl., 1949, v84, 1.

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