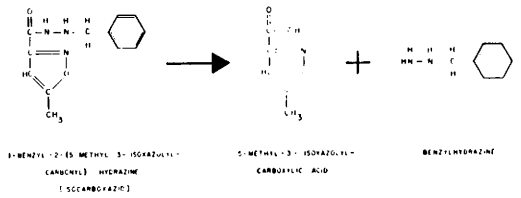

FIG. 3. Primary reaction in *in vitro* metabolism of isocarboxazid.

hydrazine. This agrees with the roughly 50% of the alcohol extract radioactivity recovered as benzylhydrazine (column b).

Discussion. It has been demonstrated *in vitro* that rat liver homogenate enzymatically catalyzes the breakdown of isocarboxazid and that one major product of this metabolism is benzylhydrazine. These findings can most simply be interpreted as reflecting a hydrolytic cleavage of isocarboxazid to yield 5-methyl-3-isoxazolylcarboxylic acid and benzylhydrazine (Fig. 3). The isolation of benzylhydrazine from animal tissues following administration of pharmacological doses of isocarboxazid has not been reported. However, Roth and Rieder† have detected benzylhydrazine in rat and guinea pig tissues following massive doses of isocarboxazid. This, in conjunction with the present finding that *in vitro* there is extensive metabolism of isocarboxazid to benzylhydrazine and the previous finding(7) that *in vivo* 75% of the drug was cleaved with the benzyl moiety further oxidized to benzoate and excreted as hippurate in 24 hours, indicates that benzylhydrazine is an intermediate in the metabolism of isocarboxazid *in vivo*. Since benzylhydrazine is a more potent MAO inhibitor than isocarboxazid, the role of benzylhydrazine as an agent responsible for various aspects of the pharmacological activity of isocarboxazid becomes of prime importance. This problem is currently being studied.

† Personal communication from Hoffmann-La Roche, Inc., Basel, Switzerland.

Summary. Isocarboxazid was extensively metabolized under aerobic or anaerobic conditions in an *in vitro* system containing rat liver homogenate as the enzyme source. Benzylhydrazine was a major product of this metabolism as determined by paper chromatography and reverse isotope dilution in studies with C^{14}-isocarboxazid. The possible occurrence of this pathway *in vivo* was discussed.

The author wishes to acknowledge the very competent technical assistance provided by Mr. E. Postma.

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Received February 6, 1961. P.S.E.B.M., 1961, v107.

Development of Serum Haptoglobins in Man.* (26705)

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Twenty years ago Polonovski and Jayle described a fraction of serum protein capable of combining with hemoglobin to form a stable complex which possessed peroxidase activity. This fraction they called haptoglobin (1). Smithies, using the technic of starch gel electrophoresis, has demonstrated that several haptoglobins may be present in a sin-

* Supported by research grants from Nat. Cancer Inst., U.S.P.H.S.

gle serum, and that there are at least 3 different genetically determined haptoglobin patterns(2). Tuttle reported that haptoglobins were not present in the blood of newborns and did not appear until after the first few weeks of life(3). Smithies observed that haptoglobins were not always present in sera of young children(2). Bearn and Franklin reported that antisera prepared from electrophoretically isolated haptoglobin complexes failed to react with umbilical cord sera, but did react with normal sera and isolated haptoglobin complexes(4). This may be interpreted as indicating the absence of haptoglobins in cord sera. On the other hand, Galatius-Jensen was able to determine haptoglobin patterns in 4 out of 34 samples of umbilical blood from newborns, and in sera of 6 of 10 children 8 days of age whose haptoglobins were not detectable at birth (5). Nymen, too, has recently reported that she demonstrated haptoglobins in 11 of 79 samples of cord blood from newborns, and could find haptoglobins in the sera of 3 infants, 2, 12, and 14 days old(6). Our preliminary investigations indicated that apparently the capacity for producing haptoglobins does not develop until some time after birth(7). In the present study we have attempted to determine more accurately the time of appearance of haptoglobins in humans.

Materials and methods. Blood was collected from the pulsating hearts of 5 fetuses, age 14, 14, 18, 18, and 23 weeks, from the umbilical cords of 61 newborn including 10 delivered by Caesarian section, and from a peripheral vein or the heel of 86 infants through 15 weeks of age. Haptoglobins were analyzed by zone electrophoresis of the serum in starch gel(8). Gels were prepared from a single batch of Connaught prehydrolyzed starch (Lot #124) using a concentration of 12.5 g of starch per 100 cc of 0.021 M borate buffer of pH 9.05. Electrophoresis was carried out at 10°C for 16 hours with a potential of 4.5 volts/cm. After electrophoresis, the interiors of the gel strips were exposed by slicing them longitudinally. One-half was stained with naphthalene black B 200 to determine the distribution of serum proteins present in the sample. The other half was stained with ortho-tolidine according to the method of Kohn and O'Kelly(9) to specifically detect haptoglobin-hemoglobin complexes and/or free hemoglobin. Except for some of the cord sera, each serum sample was run in duplicate, with and without addition of normal human hemoglobin at a final concentration of 150 mg %. Thus each sample was tested twice; once free of hemoglobin and once with hemoglobin added. When haptoglobins were present, the sample with the hemoglobin added showed a change in mobility of the bands in the haptoglobin region after staining with naphthalene black. After staining with ortho-tolidine, only the hemoglobin-haptoglobin complex and in some instances the excess free hemoglobin appeared. When haptoglobins were absent, only a free hemoglobin band was found(10).

Results. Haptoglobins were not detectable in the 5 fetal sera, and when hemoglobin was added to the samples, only a free hemoglobin band appeared. A single band present in the haptoglobin region of one sample when it was run initially, disappeared when it was run with the hemoglobin added (Fig. 1). We have found haptoglobins in 10% of the 63 cord bloods tested. Forty of them were compared to the corresponding maternal pattern, and the most significant differences were found in the haptoglobin region. In all but 6 cord sera, haptoglobins were absent, whereas all maternal patterns showed haptoglobins (Fig. 2). Haptoglobins when present in the cord sera were in low concentration as determined by visual evaluation. None of the 10 cord bloods obtained from newborns delivered by Caesarian section contained haptoglobins. The results of the electrophoretic study of the serum proteins of the 86 infants (Table I) strongly indicated that while in most instances appreciable amounts of haptoglobins may not be present at birth, they do appear as early as 12 hours after birth, are present in 75% of the infants during the first 2 weeks, and were found in 100% of the infants tested by the beginning of the third week. Where it was possible to make a serial study of the same child, the progressive increase in amount of

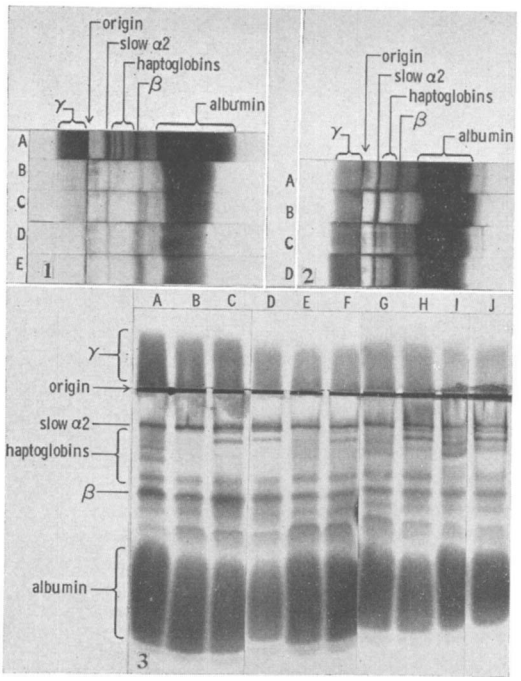

FIG. 1. Starch gel electrophoretic patterns of fetal serum proteins. *Key:* A, Adult serum. B, Fetal serum (18 wk, 16.5 cm). Proteins are seen in albumin, beta globulin, slow alpha$_2$ globulin and gamma globulin regions. Note band in haptoglobin region. C, Fetal serum (B with 150 mg % hemoglobin added). Same pattern as in B except that the band in haptoglobin region is absent and a free hemoglobin band appears just faster than beta. D and E, Fetal sera (14 wk, 11.5 cm). Pattern same as in older fetus B except for absence of gamma globulin. No evidence of haptoglobins in either sera.

FIG. 2. Starch gel electrophoretic pattern of maternal and corresponding cord serum proteins. *Key:* A, Maternal serum. B, Umbilical cord serum from child of A. No evidence of haptoglobins. All other serum proteins present. C, Maternal serum. D, Umbilical cord serum from child of C, showing haptoglobins present.

FIG. 3. Series of starch gel electrophoretic patterns of serum proteins of same child from 2 days through 22 days of age, showing progressive increase in amount of haptoglobin. *Key:* A, Adult serum (haptoglobin type 2-1). B, Infant serum (2 days) with 150 mg % hemoglobin added. There is a faint trace of haptoglobin present. C, Infant serum with 3+ hemolyses (5 days). Haptoglobins can now readily be seen. D, Infant serum, 4+ hemolyses (7 days). No change from previous haptoglobin pattern. E, Infant serum (13 days). F, Infant serum (13 days) with 150 mg % hemoglobin added. The haptoglobin pattern has become more complex with additional faster bands appearing. G, Infant serum (14 days). Still more intense pattern as compared to E. H, Infant serum with 150 mg % hemoglobin added (14 days). Haptoglobin bands now very intense. I and J, Infant serum (22 days) with no hemoglobin and 150 mg % hemoglobin added respectively. A still more intense haptoglobin pattern is shown which remains at this level as the child increases in age. The haptoglobin type is now readily discernible and can be classified as the homozygous 2-2 type.

haptoglobins with age could readily be seen (Fig. 3).

Discussion. From these observations we conclude that haptoglobins are rarely present at birth, and then only in low concentrations. This absence or low concentration of haptoglobins may be attributed either to the low rate of synthesis in the newborn, or to the fact that large numbers of red blood cells are destroyed shortly after birth, resulting in liberation of excessive amounts of hemoglobin into the plasma causing removal of the haptoglobins faster than they can be synthesized. The role played by each of these mechanisms requires further study. It seems likely that in some infants haptoglobin synthesis begins before birth, and that by the age of 2 weeks virtually all infants are able to produce haptoglobins.

Other proteins are known to develop at or shortly after birth in animals. For example, an abrupt increase in activity of tyrosine-alpha-ketoglutarate transaminase in rat livers from the second to the twelfth hour after birth has been demonstrated(11). Haptoglobin synthesis in the human precedes by a wide margin the development of antibody producing activity(12). The appearance of haptoglobins, though shown to occur somewhat earlier than previously reported, does not seem to parallel the development in man of adult hemoglobin, with which it is closely associated(13).

Definitive information is lacking regarding the site of synthesis of the haptoglobins. Lack of correspondence in the appearance of haptoglobins with development of active immune response suggests that the antibody producing cells are not a site of haptoglobin synthesis. On the other hand, the liver has not been eliminated from consideration by the older studies which utilized less sensitive electrophoretic methods. Although electrophoretic studies have indicated that at time of birth the liver is capable of synthesizing all of the major plasma fractions except gamma globulin(14), minor components can

TABLE I. Chronology of Appearance of Haptoglobins in Fetal, Newborn, and Infant Sera as Indicated by Starch Gel Electrophoresis after Staining with Ortho-Tolidine and Naphthalene-Black B-200.

Age	No. of samples	No. with haptoglobins present: Detected with: Both ortho-tolidine and naphthalene black	Ortho-tolidine stain only	Naphthalene-black stain only	% of samples containing haptoglobins
Fetal	3	0			0
Cord	63	6			10
Day of birth	2	1			
1 day	3		2		
2 days	5	4		1	
3 "	3	3			
4 "	6	5			75
5 "	6	5			
6 "	3	2			
7 "	1	1			
2nd wk	7	4			
3rd wk	3	3			
4th "	6	6			
5th "	3	2		1	
6th "	3	2	1		
7th "	10	9	1		100
8th "	6	6			
9th "	4	4			
10th "	4	4			
11th "	2	2			
12-15th wk	9	9			

be detected only by more sensitive methods.

Summary. To determine the time of appearance of the haptoglobin serum proteins in the developing human being, starch gel zone electrophoresis was performed on sera from 5 fetuses, 63 umbilical cord bloods, and 86 infants. Haptoglobins were absent in sera from the fetuses, but present in 10% of the umbilical cord bloods at birth, in 75% of the sera from infants up to 2 weeks old, and in 100% of the sera from the infants over 2 weeks of age.

For cooperation and assistance in securing the cord and newborn bloods necessary for this study, we wish to thank Dr. Marion Erlandson and Dr. Margaret Hilgartner, Dept. of Pediatric Hematology, New York Hospital, Dr. Roy Bonsnes and Miss Dorothy Wendel, Central Laboratories of New York Hospital, and Dr. Wha Shim Han, Bellevue Hospital. We also wish to thank Dr. Bartholomeus Hoogstraten, Mt. Sinai Hospital, for obtaining the fetal bloods. Dr. German Castillo, Univ. of the Philippines, participated in the early phases of the study.

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