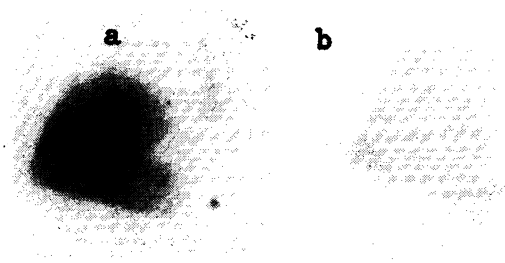

FIG. 2. Radioautographs of two successive sections of rat liver cut from the same block and exposed simultaneously; a differs from b in having no contact with water during the procedure of mounting the tissue on the slide. (Enlarged approximately 4 ×.)

night; clearing in xylol and embedding in paraffin. The series (method C) used for the section shown in Fig. 1c was as follows: alcohol-formalin (9:1), 2 hr; three changes of dioxane (2 hr, 1 hr, 1 hr); followed by the usual paraffin infiltration and embedding. The method of cutting and mounting sections from paraffin blocks was the same with all three sections shown in Fig. 1 and will be described. All three sections were mounted on the same slide and the slide was used for making the radioautographs, which are also shown in Fig. 1 (a′, b′, and c′). The exposure was made by clamping the slide against the emulsion of an Eastman Kodak NTB3 Nuclear Track Plate. The exposure time was 48 hr and was started 14 days after the injection of the animal. This newly developed emulsion is very satisfactory for obtaining radioautographs with P^{32} because of its high sensitivity to high energy beta particles. It can be seen that the amount of P^{32} retained by the tissue, as judged by the radioautographs, is not markedly different in the three methods of fixation and dehydration. However, measurements were made of radioactivity present in the solutions used in preparing tissues by method B and it was found that roughly 25% of the activity present in the tissue was lost to the solutions. This loss was less for method C (i.e., dioxane is preferable to the use of alcohols for dehydration), and in method A (the freezing and vacuum dehydration technique) no loss was possible. These three methods were chosen specifically to minimize the loss of P^{32}. While no data are available, the use of other methods (in particular the use of acid fixatives or prolonged periods of contact with solutions) would probably result in greater loss.

The method of cutting and mounting the sections was found to be very important. It is again desirable to avoid, if possible, contact with all solutions which will extract the P^{32}. This has been accomplished as follows: The sections are cut at the usual thickness of about 8 μ. Before cutting each section, the surface of the block is coated with a thin layer of melted paraffin. After this has cooled for a few seconds the section is cut; allowing the proper cooling time will avoid compression of the paraffin or curling, which occurs if the time is either too short or too long. This layer of paraffin serves as a backing for the section and has avoided the difficulties with curling and folding of the section which occur with a 8-μ section which has no backing and has not been floated on water for flattening. The section may then be mounted directly on a slide and the paraffin removed with xylol or not, as desired. The sections shown in Fig. 1 and in Fig. 2 were cut and mounted in this way on slides coated with a small amount of egg albumen; the slides were placed in the oven (40° C) for a few minutes before removal of the paraffin with xylol. In order to avoid the possibility of chemical action of the tissue on the emulsion, a thin piece of cellophane was placed between the tissue and the emulsion before they were clamped together. Fig. 2 shows radioautographs obtained from two successive sections cut from the same block of frozen, dehydrated liver and exposed simultaneously to the same plate (this was also a 48-hr exposure to an NTB3 emulsion). The two sections were different only in the following respect: after cutting, section 2b was placed on a dish of water with the tissue in contact with the water surface for a period of time judged to be similar to that used in the regular procedure when the ribbon is floated on water before mounting. The marked difference in the density of the radioautographs in Fig. 2a and 2b demonstrates the loss of a large fraction of the P^{32} by floating the section on water.

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On the Relationship of Blood Group A to Rh Immunization and the Occurrence of Hemolytic Disease of the Newborn

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In the course of an analysis of immunological data collected from 11,649 pregnant women during the years 1943–1948 inclusive, there was observed a larger number than expected of individuals of blood group A among the sensitized Rh-negative women who bore infants afflicted with hemolytic disease of the newborn. The question was then raised as to whether a relationship could be established between the presence of group A in the red blood corpuscles and an increased incidence of response to immunization on the part of an Rh-negative woman bearing an Rh-positive fetus; and secondly, whether the presence or absence of hemolytic disease of the newborn could in part be related to the ABO blood group of the Rh-positive infant.

The ABO blood group frequencies of the sensitized Rh-negative women and their offspring were compared with the distribution of ABO blood groups in a random sample of the population of this area. In order to determine

whether the four ABO blood group frequencies in the category of sensitized Rh-negative women showed statistically significant variations from the normal blood group frequencies, the data were submitted to the chi-square test and the resultant values are listed with the probabilities of their occurrence by chance alone (Table 1). The magnitude of increase of blood group A subjects among sensitized Rh-negative women who bore afflicted infants is one which might be expected to occur by chance alone only twice or thrice in 100 similar samples. It is therefore reasonable to assume that factors other than chance may be responsible for the production of this deviation. The variations in blood group frequencies among sensitized Rh-negative women bearing normal infants (Rh positive or Rh negative) showed no significant variation from the normally occurring distribution of blood groups. These data demonstrate that proportionately more women of blood group A showed Rh sensitization and were delivered of infants suffering from hemolytic disease of the newborn than women of the blood groups O, B, or AB.

Chown (*2*) analyzed the blood groups of 46 mothers of erythroblastotic children and found among them "a significantly greater number of Group AB women and a significantly smaller number of Group B." The frequency of group A in his series was normal. Cappell (*1*) found a significantly higher frequency of group A individuals among sensitized Rh-negative women. Our data differ from those of Cappell in that the significant preponderance of group A subjects occurs among those Rh-negative women who showed laboratory evidence of Rh sensitization and gave birth to infants afflicted with hemolytic disease of the newborn.

When the ABO blood group frequencies in the three categories of infants were compared with the normal frequencies, no statistically significant differences were observed. However, in the sample of Rh-positive normal infants there is a suggestive decrease in group A (29.9%) and an increased incidence of blood group O (53.2%) individuals among them, although the increase is not statistically significant. In the small sample (26) of Rh-negative normal infants, a decrease in group O (23.1%) and an increase in group A (53.8%) occurs. It may be that the sensitized Rh-negative women, who bore the latter group of infants, originally formed Rh antibodies in response to a previous exposure to Rh-positive blood and therefore are to be considered as examples of the "carry-over" or anamnestic phenomena. The data indicate that an Rh-negative blood group A or B fetus *in utero* may stimulate an anamnestic rise of Rh antibodies in an already sensitized woman more readily than a fetus of group O; but this requires further confirmation.

TABLE 1

ABO Blood Group Frequencies in Sensitized Rh-Negative Women and their Offspring Arrayed According to the Clinical Condition of the Child

Categories		Rh positive, hemolytic disease of newborn		Rh positive, normal child		Rh negative, normal child		Totals and average %		ABO blood group in random sample of population
	n =	124		78		26		228		5175
		n	%	n	%	n	%	n	%	
Sensitized Rh-negative women	O	43	34.7	36	46.2	11	42.3	90	39.5	44.8%
	A	66	*53.2*	32	41.1	10	38.5	108	47.4	39.7%
	B	11	8.9	7	8.9	4	15.4	22	9.6	11.3%
	AB	4	3.2	3	3.8	1	3.8	8	3.5	4.2%
	χ^2 =	*9.53*		0.46		0.47		5.64		
	p =	*0.03–0.02*		0.95–0.90		0.95–0.90		0.20–0.10		
	n =*	97		77		26		200		
		n	%	n	%	n	%	n	%	
Children of sensitized Rh-negative women	O	50	51.5	41	53.2	6	23.1	97	48.5	
	A	39	40.3	23	29.9	14	53.8	76	38.0	
	B	7	7.2	12	15.6	4	15.4	23	11.5	
	AB	1	1.0	1	1.3	2	7.7	4	2.0	
	χ^2 =	4.72		5.87		5.19		3.07		
	p =	0.20–0.10		0.20–0.10		0.20–0.10		0.50–0.30		

* The differences in numbers of sensitized Rh-negative women and children born to them are due to lack of information concerning the ABO groups of 28 infants.

In summary, in a sample of 228 sensitized Rh-negative women, 124 bore children afflicted with hemolytic disease of the newborn. Of these 124 women 66 (or 53.2%) belonged to blood group A. The chi-square test on these data demonstrate that they are of probable statistical significance ($p = 0.03$–0.02) and the inference may be made that this unusual increase in the incidence of blood group A mothers is not due to chance alone. These data indicate a possible relationship between blood group A of the mother and the character of her immune response to the Rh factor as antigen. The infants afflicted with hemolytic disease of the newborn showed a normal distribution of ABO blood groups.

References

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