Myocardial Changes Associated with Icterus Gravis of the Newborn

III. Results of Immune Haemolytic Experiments

By

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In newborn babies dead with icterus gravis necropsy mostly reveals an extensive hydropic vacuolar myocardial degeneration [1, 2]. In our previous studies haemolysis was induced by phenylhydrazine in infantile rabbits and degenerative myocardial changes similar to those observed in newborns dead with icterus gravis have been found. It has been assumed that hypoxia due to haemolysis was responsible for the change. In the course of these experiments no convincing proof has been obtained as to the protective effect of corticoids [3, 4].

The experiments carried out with phenylhydrazine are not comparable with human immune haemolytic disease. Since the aim of the investigations is to prevent the myocardial degeneration leading to death, and since icterus gravis is the consequence of an immune haemolytic process, in the present experiments an attempt has been made to induce lesion by an immune haemolytic process.

Material and Method

Twenty guinea pigs, 30 suckling infantile rabbit originating from 5 litters, and 4 adult rabbits ranging in weight from 270 to 480 g were used. The guinea pigs were sensitized by administering intraperitoneally doses of 0.5 ml rabbit blood on 5 occasions in intervals of 4 days. Guinea pig serum thus obtained which at a dilution of 1 : 1024 or more caused haemolysis of rabbit red corpuscles was then used in the experiments. Of this serum, 1 ml doses were administered intravenously to the infantile rabbits in intervals of 2 to 3 days. One rabbit died spontaneously following the first dose of serum, 6 animals died after the second dose, 14 rabbits were killed with air embolism 6 hours after the third dose. Nine untreated infantile rabbits from the same litter as the test animals served as controls. RBC and haemoglobin value were determined. Statistical evaluation was done with Student's test.

The heart, lung, liver, spleen, kidney and brain were studied histologically after staining with haematoxylin-eosin, May-Grünwald-Giemsa's dye, Sudan III and Best's carmine.

Results and Comment

Initial mean RBC was 4.8 million, haemoglobin 11.5 g per 100 ml; at the end of the experiment these values were 2.2 million and 6 g per 100 ml, respectively (Table I).

TABLE I

Rabbit No. Test animals	weight, g	RBC		Haemoglobin g per 100 ml		Guinea pig serum, ml
		initial	at end of experiment	initial	at end of experiment	
1.	320	4.5		11.2		1×1
2.	285	5.8		9.7		2×1
3.	270	5.9	3.2	12.1	7.7	3×1
4.	410	5.8		11.5		2×1
5.	405	5.8		10.9		2×1
6.	325	5.2		9.4		2×1
7.	450	4.	2.	13.	4.8	3×1
8.	400	4.1	2.1	13.5	5.1	3×1
9.	460	4.	2.2	11.4	5.7	3×1
10.	480	4.	1.9	11.2	5.1	3×1
11.	465	4.2		13.		2×1
12.	440	4.5		10.2		2×1
13.	410	5.	2.0	10.8	6.7	3×1
14.	405	4.8	1.6	9.6	6.	3×1
15.	480	5.2	1.6	10.8	6.7	3×1
16.	420	4.5	1.4	9.1	6.4	3×1
17.	385	5.	3.6	13.8	5.6	3×1
18.	400	5.4	3.	12.2	5.7	3×1
19.	400	4.5	1.6	12.4	5.7	3×1
20.	366	4.6	1.2	11.2	6.8	3×1
21.	415	5.	3.5	14.1	6.5	3×1
Mean		4.8	2.2	11.5	6.0	
Significance		$p < 0.001$		$p < 0.001$		

Comparison of mean initial RBC and haemoglobin values in the 14 animals with those of the spontaneously died 6 animals revealed no significant difference (RBC, $t = 1.41$, $p > 0.05$, haemoglobin, $t = 1.52$, $p > > 0.05$).

Comparison of mean initial RBC and haemoglobin values with those at the end of the experiment revealed highly significant differences (RBC, $t = 13.17$, $p \ll 0.001$; haemoglobin, $t = 11.42$, $p \ll 0.001$).

At gross examination the spleen of treated animals was hyperaemic and about three times larger than

Death		Histological findings			
air embolism	spontaneous	heart	liver	spleen	kidney
	+	V±	EH+	anaemic	—
	+	V±	EH+	hyperaemic	tubular degeneration
+		V+	EH+	hyperaemic	tubular degeneration
	+	V+	EH+	hyperaemic	tubular degeneration
	+		EH+	hyperaemic	tubular degeneration
	+	V+	EH+	hyperaemic	tubular degeneration
+		V±	—	hyperaemic	—
+		V+	—	hyperaemic	—
+		V+	EH±	hyperaemic	tubular degeneration
+		V+ myocarditis	EH+ necrosis	hyperaemic	necroses
	+	V+	—	hyperaemic	tubular degeneration
	+	V+ myocarditis	minor necrosis	hyperaemic	necroses
+		V±	—	hyperaemic	tubular degeneration
+		V+	focal inflammation	hyperaemic	tubular degeneration
+		V+	—	hyperaemic	—
+		V+ myocarditis		hyperaemic	tubular degeneration
+		V+ myocarditis	EH±	hyperaemic	tubular degeneration
+		V+	—	rich in cells	tubular degeneration
+		V±	—	rich in cells	—
+		V+	—	hyperaemic	tubular degeneration
+		V+	—	hyperaemic	tubular degeneration

V = vacuolation
EH = Extramedullary haemopoiesis

that of the untreated controls. Anaemia of the other organs was conspicuous at gross examination already. A few animals displayed icteric organs.

The heart muscle showed vacuolation in both ventricles, but especially in the left ventricle. The vacuoles contained no fat (Figs. 1, 2, 3, 4).

The brain was oedematous. In the kidneys hyperaemia and congestion in the glomerular loops and tubular necrosis were revealed. The cortical substance was anaemic and focal cortical necrosis developed in two animals (Fig. 5). In the spleen mark-

ed hyperaemia and disintegrated red corpuscles were conspicuous (Fig. 6). In the liver of most treated animals haematopoietic centres were present between the hepatic cell trabecules. This change has not developed under treatment with phenylhydrazine (Fig. 7).

The results make it seem that we have succeeded in producing a process similar to the haemolytic disease of newborns; it is hoped that the method will allow a more detailed insight into the development of the condition.

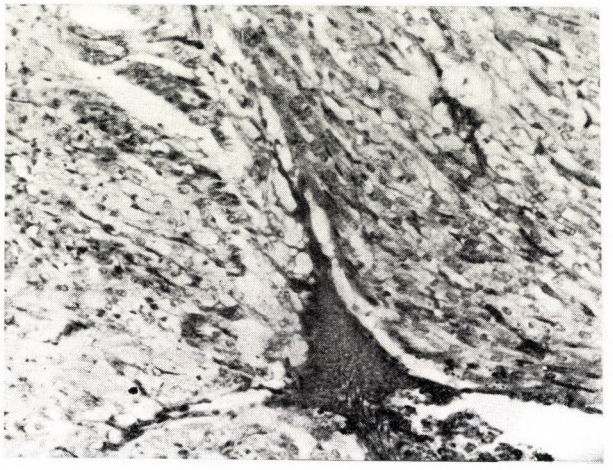

FIG. 1. Vacuolated subendocardial fibres in wall of left ventricle. H + E

Control animals

1.	370	5.9	4.0	12.4	12.4	—
2.	330	5.4	4.6	11.2	12.7	—
3.	370	5.8	4.8	10.6	10.6	—
4.	350	4.4	4.0	5.9	6.2	—
5.	310	5.0	4.1	6.4	6.5	—
6.	400	5.1	4.4	9.9	11.2	—
7.	460	5.2	4.0	10.2	11.8	—
8.	420	4.8	4.7	12.2	12.2	—
9.	340	4.0	4.5	11.7	12.2	—
Mean		5.1	4.4	10.0	10.6	
Significance		$p > 0.05$		$p > 0.05$		

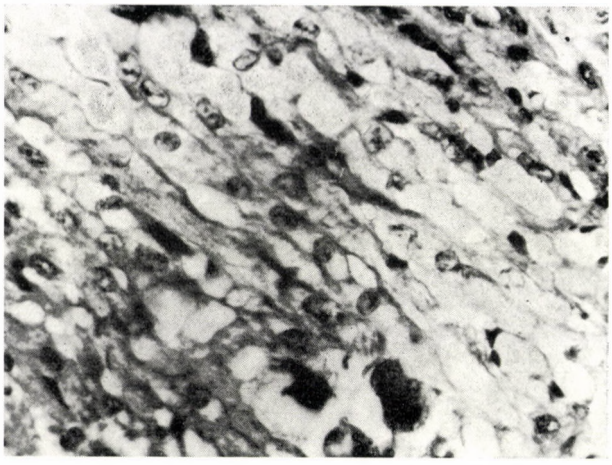

FIG. 2. Same as FIG. 1., under high power

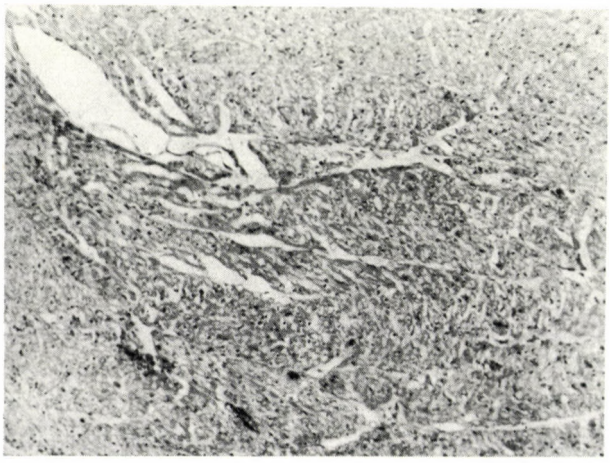

FIG. 3. Extensive vacuolation in wall of left ventricle. H + E

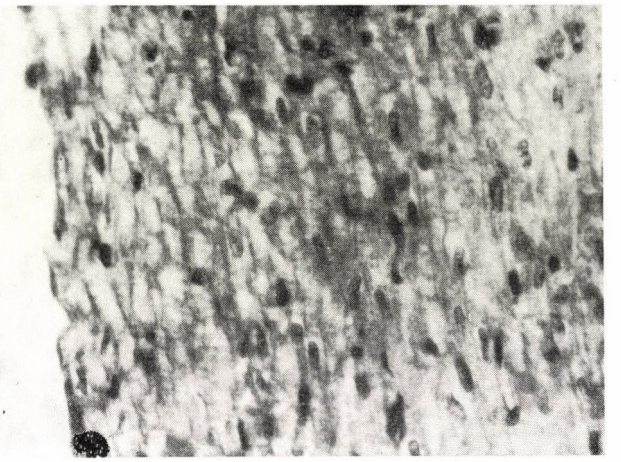

FIG. 4. Vacuolation of subendocardial fibres. H + E

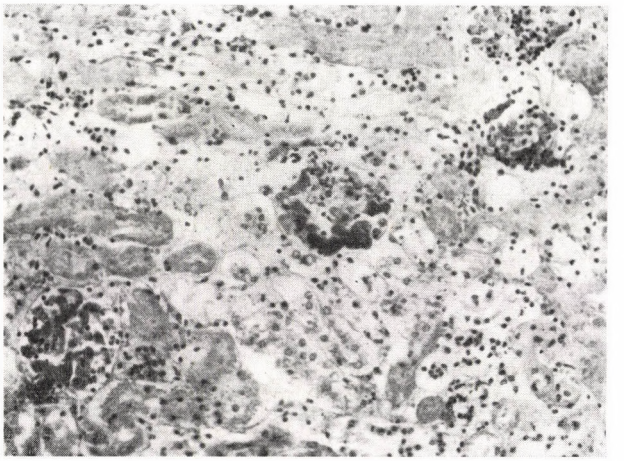

FIG. 5. Kidney. Hyperaemic congested glomerular loops, necrosed tubules, signs of inflammation. H + E

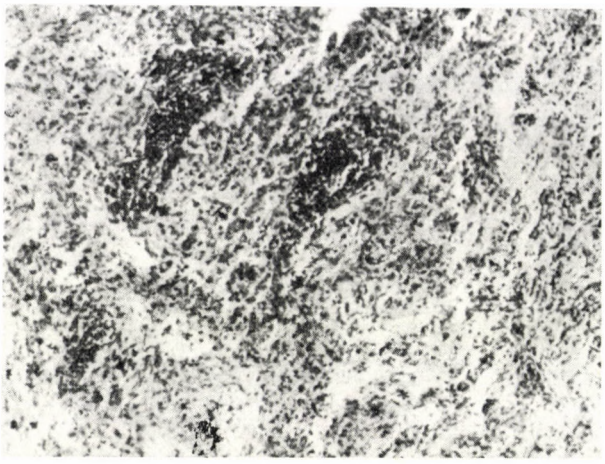

FIG. 6. Spleen. Atrophied follicles, hyperaemic pulp rich in cells. May-Grünwald-Giemsa's dye

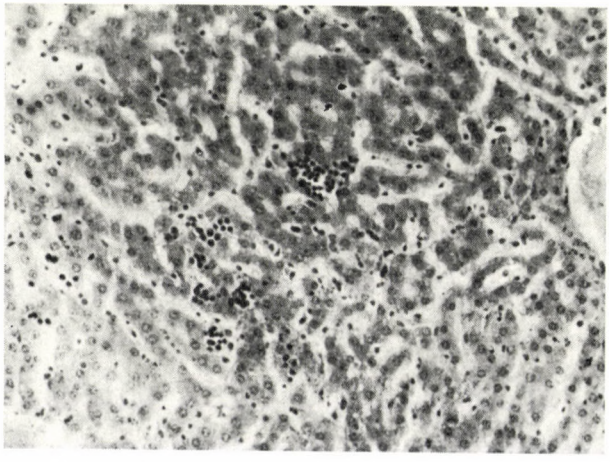

FIG. 7. Haematopoietic islets in liver. H + E

ACKNOWLEDGEMENT

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SUMMARY

In infantile rabbits haemolysis was induced by the administration of serum from guinea pigs previously sensitized with rabbit blood. As a result of the serum treatment, vacuolar hydropic myocardial degeneration developed, a change similar to that observed in newborn babies dead with icterus gravis.

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