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THE ETIOLOGY AND PROPHYLAXIS OF INFECTIOUS HEPATITIS

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ABSTRACT

Preparing Institution: Department of Biologics Research
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The incidence of hemagglutinins to monkey erythrocytes was surveyed in over 1,000 sera from normal, non-IH liver disease and IH disease donors. It was confirmed that there is no significant difference in incidence or titer of warm or cold agglutinins that would distinguish infectious hepatitis from control specimens. In a second phase of this study, methods for inactivating the non-specific agglutinins (NSA) were developed in order to determine if the NSA masked a disease-specific agglutinin. It was then found that approximately 35% of acute IH or SH specimens did contain a masked agglutinin in contrast to approximately 8% of control specimens. Attempts are in progress to increase the sensitivity of the test to masked agglutinins and to neutralize them with convalescent serum.

Numerous attempts were made to isolate virus from acute IH serum specimens using cultures from a variety of primary human tissues. Although cellular changes were observed on several occasions, in no case was the effect transmissable beyond the fourth passage. During this phase of study, a toxin was observed in fresh serum from approximately 90% of patients with acute liver disease whereas normal controls were negative. Properties of the toxin are reviewed.

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INTRODUCTION

Since there is neither a susceptible experimental animal for infectious hepatitis nor a known virus sensitive tissue culture system, and since we cannot be certain(without verification in man) that the specimen from which isolation is attempted does in fact contain the virus, a search for useful tools for examining this problem has been our first concern. If methods for detecting the agent can be derived, one unknown will have been eliminated, and the search for a suitable culture system can proceed with some degree of rationality. Under contract between the U.S. Army Research and Development Command and Chas. Pfizer and Company, two primary approaches to this problem were initiated; the isolation and propagation of icterogenic agents utilizing primary human tissue culture, and research on the immunology of infectious hepatitis with particular reference to the characterization of antigens associated with viremia. The following report describes our experience with serum specimens from patients diagnosed as acute infectious or, in a few cases, serum hepatitis. These materials have been obtained from the Milwaukee Blood Bank through the generous assistance of Dr. Ned Maxwell; from Dr. Morris Schaeffer of the New York City Health Department; and, in a large part, from Dr. Allan G. Redeker of the University of Southern California.

I. ISOLATION ATTEMPTS

A. Preparation of Cell Cultures - A determination was made of the tissue culture growth requirements of a variety of primary human tissues, particularly human kidney, lung, thymus, intestine, tonsil and placenta. Although several attempts were made to propagate liver tissue, success was only attained when the tissue was grown in the presence of one particular batch of unfiltered fetal calf serum. When that batch of serum was exhausted, we were not successful in propagating liver tissue, although 25 other serum lots were tested.

Tonsillar tissue was found to grow readily. As expected, the incidence of latent viruses in that system was so high (90%) that its use became impractical. Cultures of tonsil that showed no initial evidence of a virus often developed C.P.E. as late as the third or fourth week. Thus this system was dropped in favor of other human tissues.

In addition to human tissues, various animal tissues were cultured, such as fetal bovine lung and kidney, as well as several from the fetal monkey. Also, as an attempt to further broaden the spectrum of tissues for virus isolation, combinations of several types of cells were made with the thought that a heterologous cell population might be more sensitive to the agent of infectious hepatitis (IH) than a homologous system.

B. Isolation Procedure - Eleven IH specimens (2-4 years after collection) were each tested in two or three cell types in a systematic blind passage as diagrammed in Table I. In addition, various dilutions of twenty-five fresh, acute IH serum specimens were each inoculated into two or three types of human cells, but in this case subcultures were performed only when cellular changes were observed. In several instances a degenerative effect was

noted through the third or fourth passage, however, no further transmissible effect was evident either after prolonged observation of the original cultures or in the subcultures.

One difficulty encountered during this phase of study was a degenerative effect caused by normal human serum used as a nutrient in control tissue cultures. Studies were made to determine whether the presence of human serum per se was activating a latent agent in the cell system or whether the serum was causing a degenerative effect that required several days to manifest itself. Since degeneration of cultures by normal serum obviously would interfere with interpretation of cellular changes which might occur in the presence of HH sera, rather extensive efforts were made to inactivate the degenerative factor (D.F.) in normal serum. In summary, it was found that D.F. was inactivated or removed from the serum by a variety of exchange resins fluorocarbon, or by dialysis. It was found, however, that when a special calf serum (agamma) was used in combination with the human serum, the D.F. was neutralized. These results are summarized in Table II. Subsequent isolation attempts were successfully continued as long as the test serum was combined with an equal volume of the agamma calf serum.

We have also attempted to confirm the observations of the Parke-Davis group who reported success in growing hepatitis virus in bovine cells. We were not able to observe cellular changes that could be attributed to anything other than possible serum toxicity in numerous isolation attempts using fetal bovine kidney or lung cultures. These changes were transmissible, however, while the inoculum was in low dilution.

C. Toxicity of Human Serum In the course of these studies, fresh serum from acute HH, SH, and non hepatitis liver disease patients was often found to exert a rapid toxic effect on human and animal cell cultures. The effect occurred within 18 hours and led to complete destruction of the cells. The toxicity titer of acute specimens varied between 1:10 and 1:800, with most in the range of 100 - 200. At least 90% of the fresh acute specimens contained a measurable "toxin". None of twenty-five fresh, normal sera had the rapid toxic effect when tested in parallel. It is not clear at this time whether the toxic effect is distinct from the degenerative factor just described. It is possible that they are the same entity but manifest themselves differently, depending upon concentration. Whereas the degenerative factor appeared several days after inoculation of cultures and caused slow progressive subtle changes, the "toxin" exerts its effect within hours, causing discrete rounding of cells, not unlike virus degeneration with rapid progressive deterioration of the culture.

Some of the properties of the "toxin" are as follows:

1. Its titer is conveniently determined by introducing dilutions of sera into bovine, monkey, human, or chick tube culture monolayers, allowing 0.2 ml inocula to adsorb approximately 3 hours. Maintenance media with no additional serum is then added and cultures incubated overnight at 37°C. in a stationary rack. The titers are easily read with endpoints considered as that dilution where normal cells are found.

2. Preliminary results suggest that the "toxin" level is highest in early disease and becomes negative sometime during convalescence.

3. Serum toxicity is inactivated by treatment with mercaptoethanol.

4. The "toxin" is inactivated by acid treatment as a function of time. At pH 2.0, the material is usually destroyed within 30 minutes.

5. Serum toxicity is not consistantly inactivated by treatment at 56°C for 30 minutes. Similarly, the toxicity of serum at room temperature falls off in 2 - 3 days whereas at 5° it is usually active for at least 2 weeks, depending on the initial titer. The retention of toxicity by frozen sera has not been fully established.

6. The "toxin" is more resistant to heat inactivation when cystine at pH 7.2 is added.

7. It is not dialyzable.

8. Toxicity is neutralized by normal human serum and by human serum albumin. It is not neutralized by gamma globulin nor by IH convalescent serum from donors up to one year post-acute phase.

D. Serum Detoxification and Dissociation - Since most fresh acute IH sera were toxic, it became necessary to pretreat the specimens to inactivate the toxin before they could be introduced into tissue culture systems for attempted virus isolation. It was also postulated that in view of the relatively long incubation period of the disease, it is possible that antibody might be present, even during the acute phase which, if in a complex with the virus, would render an isolation attempt more difficult. Procedures were therefore studied for pretreating serum specimens to be used for virus isolation which would inactivate serotoxicity and also release virus from a possible antibody complex. These have included treatment with fluorocarbon, enzymatic digestion, acid treatment, and exposure to mercaptoethanol. The final method selected for "toxin" inactivation and antibody dissociation must be based not only upon its effectiveness for those two purposes but also must not be harmful to the potential IH virus in the specimen. We have therefore subjected two enterovirus models to these conditions on the assumption that if the enterovirus models survive the treatment, the probability that hepatitis virus would also survive is increased. The results of this study showed that not only are poliovirus I and ECHO 1, resistant to acid, mercaptoethanol, fluorocarbon, and enzymatic treatments in terms of infectivity or hemagglutinating ability in the case of ECHO 11, but their titers were often increased as much a one log following these treatments. In view of our successful inactivation of serotoxicity by acid, and the reported dissociation of enteroviruses from antibody by acid (Mandel 1958, Pinkeiro and Hsiung 1963), we are now employing acid as our method of choice for pretreatment of specimens.

II. IMMUNOLOGICAL STUDIES - UNTREATED SERUM

A. Analysis of Untreated Serum - The agglutination of erythrocytes from several animal species by serum from patients with infectious hepatitis has been the subject of several previous reports including Hoyt and Morrison (1956), Rubin (1957), Havens (1958), McCallum (1959) and Jennings and Hindmarsh (1960). Although little hope previously has been given to the agglutination

reaction for detecting hepatitis virus, several unanswered questions have encouraged us to restudy this phenomenon with the following objectives:

1. To determine if there is a significant difference in IH and non-IH reactors using previously described or modified techniques;

2. To determine if more than one type of agglutinin is involved,

3. To evaluate erythrocytes from various species for sensitivity to the agglutinin,

4. To review characteristics of the agglutinin.

B. Agglutination Test Procedure - The test was performed by combining 0.5 ml of doubling dilutions of serum in saline with 0.2 ml of a 1% washed erythrocyte suspension. Serum dilutions of 1:10 through 1:320 were ordinarily tested. The cells and serum were combined in acid cleaned Wasserman tubes, incubated one hour at 37°C, observed microscopically for agglutination, re-incubated at 4° for two hours to overnight, and reread microscopically for evidence of cold agglutinins. As little agglutination as firmly paired red cells was regarded as a positive.

C. Source of Serum Specimens - Serum was collected from children and adults of several age groups and were of six general catagories. normal children, mentally defective children, normal adults, non-IH liver diseased adults, hepatitis diseased adults, and hepatitis convalescent adults. Serum from the non-liver diseased populations, ordinarily were pre-immunization specimens from measles or influenza vaccine field trials which had been stored up to two years at 4°C. The majority of the hepatitis specimens had storage histories of one month or less.

D. Results of Agglutination Test - Agglutination of monkey erythrocytes by normal and IH sera was found to be temperature dependent in that both warm and cold agglutinins were observed. Results of these studies summarized in Tables IIIA, IIIB, and IV permit the following conclusions:

1. Warm Agglutinins -

   a. Children (6 months 6 years) whether normal or mentally defective have the same incidence of agglutinins with approximately 75% reacting at a serum dilution of 1:10.

   b. Adults in the middle age bracket (18-40), whether in an acute or convalescent stage, show a reduced incidence of warm agglutinins with an average of 65% reactors at a dilution of 1:10.

   c. Adults in the older age group (50-80) show a marked difference in incidence of warm agglutinins with only approximately 10% reactors at a serum dilution of 1:10.

In general, the incidence of warm agglutinins appears to be unrelated to hepatic disease, with any significant differences in reaction rate being age dependent. A similar observation was noted by Jennings and Hindmarsh (1960).

2. Cold Agglutinins

   a. All categories except the older population had virtually 100% incidence of cold agglutinins at a serum dilution of 1:10,

The older group was remarkable in that 57% showed no measurable cold agglutinins.

b. In addition to the high incidence of cold agglutinins, 66% of the young population had titers of 1:160 or higher, whereas 38% of the middle age adult population, whether normal or pathological, had agglutinins at the 160 level. The older population again differentiated sharply with only 7% showing titers as high as 1:40 with the majority in the 1:10 or <1:10 range.

c. As shown in Table IV, the incidence of cold agglutinins only may be higher in convalescent IH and SH patients than in other groups, with 40 and 45%, respectively, reacting. In contrast, reaction rate in the young and middle age populations was in the range of 20%. Although the incidence of cold agglutinins in the older population approaches 40%, it is undoubtedly age dependent. The possible relation of increased cold agglutinins with convalescence is strengthened by the results of the non-IH liver diseased group, where again the incidence is in the same range as adults with acute hepatitis and with levels in younger populations.

The outstanding feature of the cold agglutinin survey was again the apparent age dependency of the agglutination incidence and titers.

E. Erythrocyte Evaluation - Red blood cells from sheep, chicken, ferret, guinea pig, dog, rabbit, and monkey were compared for their sensitivity to agglutinins in serum from normal, non-IH liver and IH liver disease specimens. The results shown in Table V may be summarized as follows:

1. Rabbit and dog cells are considerably more sensitive to warm and cold agglutinins. The descending order of sensitivity for warm agglutinins is: rabbit; dog; guinea pig; monkey; sheep; chick; and ferret. For cold agglutinins, the order is: rabbit; dog; guinea pig; ferret; monkey; sheep; and chicken.

2. In general, specimens with low agglutinin levels to rabbit or dog had little or no agglutinin for other species. Conversely, those with very high titers (5000+) to rabbit had measurable activity against other species.

3. The ferret cell was essentially non-reactive to warm agglutinins, but was agglutinated by cold agglutinins.

4. In a comparison (not tabulated) rhesus, cynomolgus, and vervet monkey erythrocytes were examined for sensitivity to warm and cold agglutinins, and no significant differences were found.

In summary, the incidence and agglutinating titer of serum from normal and pathological donors is dependent on the laboratory procedure and the species of erythrocytes employed. There is little to suggest that any of the results can be successfully used to distinguish normal from IH sera.

F. Properties of the Agglutinins - The following properties of the non-specific agglutinin have been observed:

1. Non-dialyzable.
2. Heat stable at 56° C. for 30 minutes.
3. Inactivated by proteolytic enzymes, mercaptoethanol and acid treatment.
4. Sedimented with macroglobulins.
5. Removed by fluorocarbons.

The sedimenting properties and mercaptoethanol sensitivity of the agglutinins suggest that they belong in the macroglobulin class. Havens (1958) observed them in the gamma globulin fraction and also noted their non-dialyzing and heat stable properties.

III. IMMUNOLOGICAL STUDIES - TREATED SERUM

A. Analysis of Treated Serum - In view of the high incidence of non-specific agglutinins in normal and IH sera, an attempt was made to determine if the non-specific agglutinins masked a specific disease-related agglutinin. Removal of agglutinins by low pH has been studied most thoroughly in our laboratory. With this procedure a survey was conducted on 164 acute IH or SH, 72 IH or SH convalescent, 5 lupoid, 27 non-IH liver disease, and 172 normal sera for incidence of agglutins to monkey erythrocytes before and after acid treatment.

Results summarized in Table VI show that IH and non-IH serum specimens do contain an agglutinin in addition to the acid labile pan-agglutinin, and that the incidence of the acid resistant agglutinin may be significantly higher in acute IH or SH serum than in control serum.

Whether the resistant agglutinins are hepatitis related remains to be established. However, several miscellaneous observations have been made which are compatable with the possibility that the acid-resistant agglutinins are disease related.

1. Where multiple acute specimens have been drawn from the same patient, we have often found two or more consecutive bloods positive for acid resistant agglutinin (ARA) becoming negative in the convalescent stage.

2. Two enterovirus models (polio-I and ECHO-11) were found to retain full infectivity and hemagglutinating properties in the case of ECHO-11 after acid treatment.

3. In a limited screen, specimens which contained ARA were found also to contain mercaptoethanol-resistant agglutinins.

4. Whereas acute IH specimens that agglutinate erythrocytes from a variety of other animal species before acid treatment, react only with monkey cells after acid treatment.

5. ARA are not related to the non-specific agglutination titer observed before acid treatment. In other words, a specimen may have a very high non-specific agglutination titer (over 1:320) and an ARA titer of 1:20 after treatment. On the other hand, a sample may have a non specific agglutinin titer of 1:40 before treatment and also show an ARA titer of 1.20 after treatment.

6. The fact that less than 100% of acute specimens contain the ARA could be due to the relative concentration of the ARA, which, in turn, could be dependent on date of onset of disease. Analyses are not complete to determine if such a correlation exists.

Further studies characterizing the acid-resistant agglutinins are in progress, as are attempts to specifically neutralize the activity with convalescent serum.

SUMMARY

Serum from normal and infectious hepatitis donors was screened for warm and cold agglutinins to monkey erythrocytes. It was found that in untreated serum, no correlation exists between the incidence or titer of agglutinins and the source of specimens. Results did confirm, however, that agglutinin levels are age-dependent, being highest in the young and lowest in the older population.

In serum pre-treated to remove the non-specific agglutinins a second "masked" agglutinin was observed in a higher percentage of acute IH or SH specimen than in control specimens. Whether the masked agglutinin is disease-related has not yet been established.

In another phase of the study, numerous isolation attempts were made using serum from acute IH patients and a variety of tissue culture systems. Although on several occasions cellular changes were noted, no effect was transmissable beyond the fourth passage.

During the course of isolation attempts, a relatively potent toxin was noted in most of the serum specimens from patients with acute liver disease. Some of the properties of the toxin were described.

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Table I.

PROCEDURE FOR BLIND PASSAGE STUDIES

Test

0 0 0 0 0 ← Inoculate 0.2 ml IH serum/tube and subculture at weekly intervals.

7-Day Sub | 14-Day Sub | 21-Day Sub

	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Blind	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Pass 1	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Blind	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Pass 2	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Blind	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Pass 3																

Controls

0 0 0 0 0 ← Inoculate 0.2 ml control serum/tube and subculture at weekly intervals.

7-Day Sub | 14-Day Sub | 21-Day Sub

	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Blind	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Pass 1	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Blind	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Pass 2	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Blind	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Pass 3																

V = Fluids pooled and 0.2 ml passed into each of 2 tubes and remainder frozen.

1 = Challenge with a strain of Coxsackie virus at end of test.

2 = " " " " Echo virus at end of test.

3 = " " " " Vaccinia or alternate virus at end of test.

4 = Reserve for staining.

TABLE II

TREATMENT OF NORMAL HUMAN SERUM TO REMOVE "DEGENERATIVE FACTOR"

Substance Tested	Results
Serum dialyzed against Carbowax	Degeneration of HK[1] cells
" " " saline pH 7.2	"
" " " saline pH 9.0	"
" genetron treated	"
" adsorbed with dried human liver	"
" " " Deminite	"
" " " Dow 2X-8	"
" " " Dow 50X	"
" " " IR 4B (OH)	"
" " " IR A-400 (C)	"
" " " IR 120 (H)	"
" " " CM-Cellulose	"
" " " CM-Sephadex	"
" " " DEAE	"
" " " Amberlite XE67	"
" " " DEAE-Sephadex	"
Untreated control	"
No serum control	Slight degeneration
Serum combined with agamma calf serum	No degeneration

[1] Human kidney

TABLE III-A

AGGLUTINATION TITERS OF NORMAL AND PATHOLOGICAL SERUM SPECIMENS

Incidence of Warm Agglutinins at Each Dilution (%) (1)

Age Group & Source of Specimens	Number of Specimens Tested	Agglutination Titer <10	10	20	40	80	160	320 (3)
Mental Defectives 6 mos. - 6 yrs.	234		78 (2)	53	34	18	6	2
Orphanage 6 mos. - 6 yrs.	39		79	53	40	20	10	5
Normal 6 mos. - 6 yrs.	439		76	38	17	8	2	0
Normal 25 - 40 yrs.	58		80	60	50	15	5	2
Normal 50 - 80 yrs.	305		10	6	2	0	0	0
IH Acute 18 - 35 yrs.	64		70	53	39	12	6	3
IH Convalescent 18 - 35 yrs.	59		57	35	16	8	0	0
SH Acute 18 - 80 yrs.	20		60	45	15	5	5	5
SH Convalescent 18 - 80 yrs.	11		54	45	9	0	0	0
Non-IH Liver Disease 18 - 80 yrs.	53		71	41	30	21	12	8

(1) % to nearest whole number; (2) Example - 78% had titers of 10 or higher; (3) Equal to or > 320.

TABLE III-B

AGGLUTINATION TITERS OF NORMAL AND PATHOLOGICAL SERUM SPECIMENS

Incidence of Cold Agglutinins at Each Dilution (%) (1)

Age Group & Source of Specimens	Number of Specimens Tested	Agglutination Titer <10	10	20	40	80	160	320 (3)
Mental Defectives 6 mos. - 6 yrs.	234	0	100 (2)	98	92	85	69	40
Orphanage 6 mos. - 6 yrs.	39	0	0	100	95	92	77	33
Normal 6 mos. - 6 yrs.	439	0	99	96	91	76	53	25
Normal 25 - 40 yrs.	58	0	100	98	95	78	52	19
Normal 50 - 80 yrs.	305	57	43	14	7	0	0	0
IH Acute 18 - 35 yrs.	64	0	100	93	80	60	41	27
IH Convalescent 18 - 35 yrs.	59	2	98	90	71	51	39	22
SH Acute 18 - 80 yrs.	20	10	90	75	60	45	30	15
SH Convalescent 18 - 80 yrs.	11	0	99	81	63	45	18	18
Non-IH Liver Disease 18 - 80 yrs.	53	2	97	88	77	64	51	45

(1) % to nearest whole number; (2) Example - 100% had titers of 10 or higher; (3) Equal to or > 320.

TABLE IV

INCIDENCE OF WARM AND COLD AGGLUTININS
IN ACUTE S.H., I.H., AND CONVALESCENT SERUM

Identification	Total No. Tested	% of Total[2] Actual Number Responding: Warm Only[1]	Cold Only	Warm Plus Cold	None
Mental Defectives 6 mos. - 6 yrs.	234	5 11	21 50	74 173	0 0
Normal - Orphanage 6 mos. - 6 yrs.	39	10 4	18 7	72 28	0 0
Normal 6 mos. - 6 yrs.	439	5 23	22 97	73 319	0 0
Normal 25 - 40 yrs.	58	3 2	19 11	77 45	0 0
Normal 50 - 80 yrs.	305	4 12	37 113	7 19	53 161
IH Acute 18 - 35 yrs.	64	6 4	30 19	64 41	0 0
IH Convalescent 18 - 35 yrs.	59	7 4	40 24	50 30	2 1
SH Acute 18 - 80 yrs.	20	15 3	25 5	50 10	10 2
SH Convalescent 18 - 80 yrs.	11	0 0	45 5	55 6	0 0
Non-IH Liver Disease 18 - 80 yrs.	59	7 4	24 14	68 40	2 1

(1) Specimens showing no rise in titer after incubation in the cold, were regarded as positive for warm only. It is recognized that both could be present at the same titer.

(2) Percent to nearest whole number.

TABLE V

AGGLUTINATION OF VARIOUS RED BLOOD CELLS BY SERUM FROM NORMAL AND LIVER DISEASE DONORS.[1]

Warm Agglutinins

	No. Tested	Chick	Dog	G. Pig	Ferret	Rabbit	Sheep	Monkey
Normal Controls	20	3.0	150	25	0	175	20	22
Non-IH Liver Disease Controls	10	2.0	96	15	3.0	420	7	10
Milwaukee IH	8	0	8	2	0	40	0	1.0
Los Angeles IH	10	2.0	90	56	0	150	0	7.0

Cold Agglutinins

Normal Controls	20	13	640	225	125	1280	150	190
Non-IH Liver Disease Controls	10	68	800	300	130	1800	100	145
Milwaukee IH	8	8	160	65	50	2000	30	30
Los Angeles IH	10	17	640	200	240	1900	115	100

[1] Agglutination titers are a composite of two tests and expressed as geometric means.

TABLE VI

INCIDENCE OF ACID-RESISTANT AGGLUTININS IN SERUM FROM LIVER DISEASE AND NORMAL HUMAN SERUM CONTROLS

	No. Specimens Tested	No. Positive Before Acid Treatment	No. Positive After Acid Treatment	Per Cent Positive After Acid Treatment
SH or IH Acute	72	72	24	33%
SH or IH Convalescent	60	60	3	5%
Lupoid	5	5	3	60%
Non-IH Liver Disease	27	25	1 *	4%
Normal 6 mos. - 6 yrs.	63	58	5	8.6%
Mental Defectives 6 mos. - 6 yrs.	46	41	3	7.3%
Normal Middle Age 18 - 40 yrs.	26	24	3 **	12.5%
Normal Older Age 50 - 80 yrs.	37	16	0	0

* Lupus erythematosis.

** Two of 3 positives work in hepatitis building.