Blood Transfusions and Serum Hepatitis: *

Use of Monochloroacetate as an Antibacterial Agent in Plasma

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Interest in serum hepatitis in the past has centered largely about its occurrence in connection with the administration of pooled plasma. With the resolution of the major problem of hepatitis transmission by pooled plasma, that from whole blood comes into sharper focus, and is the subject of this report. In addition, experimental data and preliminary clinical experience with the use of the sodium salt of monochloroacetic acid as a means for controlling bacterial contamination in plasma or blood are also summarized in this presentation.

I. Serum Hepatitis from Whole Blood Transfusion When No Plasma is Administered

A. Source and Character of Data Comprising Study

The data which follow were obtained from a survey of patients receiving blood transfusions without plasma at the University of Chicago Clinics during the years July 1, 1946, through December 21, 1956. Because there were 11,382 patients who received blood during this ten-and one-half-year period, the magnitude of follow up for the entire group was too large to be restudied with the appropriate care required to obtain data of sufficient accuracy to answer satisfactorily the many questions that serum hepatitis from blood presented. Therefore, a random sampling technic was devised by Professor Shirley Star of the National Opinion Research Center. This resulted in a final selection of approximately 21 per cent of the total group of patients who received blood only (without plasma, etc.) during these ten and one-half years. This sample designated by unit number some 2,388 patients to be retraced.

Of the 2,388 patients in the sample, 206 died before the 30th day following transfusion; 243 died between the 30th and 180th days, and 45 could not be traced. In all, the clinical outcome was successfully established in 98.1 per cent of the patients falling into the sample. Deaths and the 45 not traced reduced the 2,388 patients to 1,894 who survived the full six months, though the fate of 2,343 patients (all but the lost 45) was also fully established. Some reassurance as to the validity of the sampling procedure employed may be gained from Table 1 in which certain points of comparison are possible between the entire 11,382 patients and the 2,388 who comprise the sample.

The diagnosis of serum hepatitis with jaundice was considered *established* when liver biopsy was performed and the pathologic findings confirmed the clinical suspicions; or when the clinical pattern was in no way in conflict with the clinical diagno-

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TABLE 1. *Total Bloods and Mean Bloods per Episode by Fiscal Year*

Fiscal Year	Distribution of Blood Only Episodes*					
	Followup Sample			Complete Record		
	Number of BO Episodes	Total Bloods in BO Episodes	Mean Bloods per BO Episode	Number of BO Episodes	Total Bloods in BO Episodes	Mean Bloods per BO Episode
1946–47	209	726	3.5	977	3,035	3.1
1947–48	206	772	3.7	897	3,114	3.5
1948–49	211	555	2.7	1,086	3,703	3.4
1949–50	242	918	3.8	1,118	4,681	4.2
1950–51	262	991	3.8	1,200	4,438	3.7
1951–52	261	939	4.0	1,159	4,015	3.5
1952–53	312	1,026	3.3	1,415	4,210	3.0
1953–54	325	1,150	3.5	1,497	4,934	3.3
1954–55	303	971	3.2	1,484	4,806	3.2
1955–56	249	777	3.1	1,211	3,855	3.2
1956 (first half)	116	340	2.9	554	1,616	2.9
Total, July 1, 1946–Dec. 31, 1956	2,696*	9,165	3.4%	12,598	42,407	3.4%

* 2,696 transfusion episodes relate to 2,492 patients in sample; of these, 54 were included in previous studies and 50 had received no transfusions, leaving a residuum of 2,388 patients for active study.

The term "transfusion episode" refers to a series of blood transfusions when multiple units were given. It also refers to a single transfusion if this was all the patient received. Specifically, this term was defined as the period of time spanned by multiple blood transfusions in which the patient received no transfusions of any kind during the five months preceding the first transfusion in the episode. The episode was terminated six months after the last transfusion had been given. Because Table 1 relates to episodes only in which 204 multiple episodes are included, i.e., 2,492 patients received 2,696 transfusion episodes. In the case of the 204 multiple episodes, the practice was to count only the first episode unless serum hepatitis with jaundice developed during a second, third, etc., episode; in these patients the episode referred to was the one in which hepatitis developed.

sis. The diagnosis was considered *questionable* when the pathologic diagnosis was not definitive or there were other conditions which may have contributed to jaundice, i.e., pre-existing liver, hepatic metastases from proven carcinoma, or biliary tract disease. Those listed as questionable cases were more likely serum hepatitis with other complications than the term may indicate. When we used this same category for classification in a similar study of patients receiving only our plasma (without blood), no questionable cases arose.[11]

The survey in its entirety was complex and included many additional bodies of data not included in this report. Each living patient was recontacted, except for the 45 who could not be located. Of the 449 who died between day one and day 180 after transfusion, the circumstances of death were established by contacting the patient's physician or a close relative. Because most of these patients continued under the care of members of the staff at the University of Chicago Clinics following their transfusion episode, their clinical course was already known to us. For those who had moved away, in some instances to foreign countries, contact often was difficult and at times taxed one's ingenuity.

B. The Hazard of the Professional Donor

The carrier blood donor constituted one of the problems we sought to appraise. It was our hope to learn more about the clinical viremic state in an otherwise healthy donor whose blood had presumably caused serum hepatitis when transfused to a patient. It is well known that blood or plasma drawn from patients sick either from serum or infectious hepatitis and transfused to others is capable of inducing serum hepa-

TABLE 2. *Incidence of Hepatitis Following Use of Professional Donors versus Family Donors*

	Patients Transfused	Hepatitis	
		No. of Cases	Attack Rate
Group I. All Blood Transfusions (Multiple and single)			
Family donor only	1,152	8	0.69%
Professional donor only	311	13	4.13%
F.D. and P.D.	431	23	5.3%
	1,894	44*	2.31%
Group II. Single Donor Recipients of Blood Only			
Family donor single unit	569	2	0.3%
Professional donor single unit	186	6	3.2%
	755	8**	1.06%

* Includes 36 established cases and 8 questionable cases.
** The 8 cases shown in Group II also appear in Group I.

titis in susceptible individuals. It is not clear, however, whether the carefully screened healthy blood donor, whose blood produces serum hepatitis in the recipient, develops the disease clinically himself, either prior to or subsequent to his donation of blood. Insofar as this incomplete study can be relied upon, none of the 437 suspect donors subsequently requestioned admited to serum or infectious hepatitis following his donation. However, one donor later acknowledged that he had infectious hepatitis 18 months earlier and thought the question about the history of jaundice was not pertinent to his state of good health at the time he presented himself as a donor for his friend. When re-interrogating donors, all questions were carefully phrased so as not to prejudice the answer.

Blood whose plasma was icteric was discarded; batteries of liver function tests were performed on many of the suspect donors when re-examined, but these, too, failed to identify the carrier-state. Thus in our experience it has not been possible to recognize the healthy carrier-donor by the methods presently available and adaptable for routine use. The carrier-donor continues to present a risk whenever blood transfusions are given. We sought to establish the magnitude of this risk our blood donors represented when their donations were transfused as whole blood. As will be shown later, this risk was less when donors were not institutionalized, or were not students in dormitories, or persons otherwise in close contact with patients who may suffer from infectious hepatitis.

Our donors came from three general sources; two types of professional donors were used, the majority of these being inmates in a local prison. The third source was the family or volunteer donor, usually a friend or relative of the patient.

Of the 1,894 surviving patients in the randomized sample receiving blood only, 36 developed serum hepatitis in whom the diagnosis was considered as *established;* eight more developed hepatitis with jaundice in whom the diagnosis was considered *questionable.* In Table 2 is shown the source of blood from which these 44 patients received their transfusions.

In Table 3 are shown the total blood donations drawn each of the ten and one-half years covered in this study. No effort

TABLE 3. *Showing Correlation of Rise in Cases of Hepatitis Per Year with the Increased Percentage of Professional Donors Used*

Year	Total Donations	Per Cent Professional Donors	Cases of Hepatitis
1946–47	4,866	0	0
1947–48	4,652	0	3
1948–49	5,031	0	2
1949–50	6,736	5	15
1950–51	5,931	26	14
1951–52	6,397	31	12
1952–53	6,813	35	37
1953–54	7,185	29	15
1954–55	8,135	50	42
1955–56	7,234	63	17
1956–57	8,112	69	32
			189

was made here to separate those whose blood eventually was converted into plasma or to separate those whose blood was transfused to the sample group. It will be noticed that all donors prior to 1950 were of the *family* type. Beginning February 2 of that year, *professional* donors were first employed, and with each succeeding year the percentage of professional donors has increased. The disappearance of the family donor has created a special problem for the University of Chicago Clinics because many of its patients are from distant geographic areas and cannot supply their own donors, leaving no recourse than to purchase blood to meet their needs.

C. *The Risk of Serum Hepatitis from Multiple Blood Transfusions versus the Single Transfusion*

In Table 3 is also listed the number of cases of serum hepatitis attributed to blood for the entire 11,382 patients transfused. The actual number of cases is somewhat greater than shown as full follow up studies were carried out only on the 2,388 patients in the randomized *blood only sample*. Extrapolating from the sample to the entire group, one may expect approximately 180 established cases and another 40 in whom the diagnosis may be held in question. The total known cases of serum hepatitis for the entire group were 189 but these have not yet been subclassified as to the certainty of diagnosis.

The percentage of patients in the randomized sample who received one or more transfusions of blood are classified accordingly and shown in Table 4. While the trend is one of a definite increase in the attack rate as the numbers of units of blood given increase up to 5 units, the attack rate thereafter does not continue to rise, presumably because of the much higher mortality prior to 30 days. In general, the highest 30-day mortality occurred in those groups of patients whose disease required larger numbers of transfusions. Hence these computations soon become spurious and do not warrant further exploration at this time.

D. *Carrier-Donor Expectancy in Healthy Donors*

The expectancy rate for encountering a carrier or a potentially infectious donor in our blood bank seems worthy of some attention. The only basis upon which such computations can be made at present entails an assumption about the expected attack rate for those patients who receive infectious blood or plasma from a common source. The attack rate for a particular infectious pool cannot be accurately assessed

TABLE 4. *Distribution of Patients in 21% Sample According to Units of Blood Given*

Blood Only	No. of Patients	% of Patients in Group	Hepatitis with Jaundice	
			No. of Cases	Attack Rate
1 unit	868	36.5%	8	1.06%
2 units	543	22.5%	13	2.78%
3 units	279	11.9%	3	1.28%
4 units	203	8.6%	5	3.14%
5 units	118	5.0%	4	4.81%
6 or more	377	15.5%	11	3.99%
	2,388	100.0%	44	

TABLE 5. *Attack Rates for Icterogenic Plasma, Serum or Blood When Recipients Completely Traced*

Material		
a. Pooled Convalescent Measles Serum		
Propert, 1938	7 out of 7	Attack rate 100%
MacNalty, 1938	26 out of 82	Attack rate 37.6%
Jervis, 1943	23 out of 80	Attack rate 30%
Ministry of Health, 1943	6 out of 14	Attack rate 43%
Gordon, 1944	79 out of 165	Attack rate 48%
b. Serum containing Yellow Fever Vaccine		
Sawyer, 1944		
1. Lot 335	351 out of 1,530	Attack rate 23%
2. Lot 338	495 out of 2,631	Attack rate 19%
c. Plasma		
Murray, 1954[1]		Attack rate 53%
Sayman, 1958	7 out of 42	Attack rate 17%
d. Blood		
Murray, 1954[2]	17 out of 75	Attack rate 22.6%

unless all patients who received their plasma or blood from the suspected source can be completely traced for a full six months following transfusion exposure.

Although there are many reports about attack rates from pooled plasma or serum, there are only a few in which it has been possible to establish the outcome of all recipients of the units administered. Those listed in Table 5 appear to meet these follow up requirements and shed light upon the attack rates that may accompany the administration of an infectious blood or a pool of plasma when given to multiple patients. It is clear, however, that the attack rate for one pool may be higher than that of another, an example of the vagaries of biologic variables—in this instance of virulence.

If the data on plasma attack rates are translated into the same terms as applying to whole blood, we may assume that a patient receiving a unit of infectious blood has between one chance in three and one chance in six of developing clinical manifestations of serum hepatitis with jaundice. Since the attack rate for *one* transfusion of blood in our hospital was 1.06 per cent (Table 2), it is reasonable to assume that two to five more patients probably received infectious blood but did not come down with the disease. Perhaps some developed subclinical serum hepatitis which this study was unable to assess.

Using the clinical cases of serum hepatitis following the administration of a single transfusion of blood without plasma as a basis for calculation, it appears that 3 to 6 per cent of our donors may have been infectious at the time they made their donations. Stated otherwise, we probably encountered one carrier-donor in every 15 to 35 healthy donors drawn. This tentative estimate is of some interest to our previous reports on the safety of our plasma. Our pools contained an average of 29 donations prepared from the outdated blood supplied from this same donor population. Our plasma was stored for six months at 31.6° C. prior to administration as a means to reduce the hepatitis virus in plasma. It seems most unlikely that a large percentage of the 539 pools prepared in this manner and dispensed did not contain plasma from one or more carrier-donors. Yet after 31.6° C. storage for six months, no cases of hepatitis have been detected; follow up for this group was 98 per cent complete.

TABLE 6. *Mortality Rate for Established Cases of Serum Hepatitis Only*

Total Cases	189
1. Early	8.3%
2. Total	13.9%

E. Mortality from Hepatitis following Blood Transfusions

In a general hospital such as our where the majority of patients transfused are in the middle or older age groups, the mortality of serum hepatitis is higher than may be experienced by other institutions in which patients of all ages are more evenly distributed. Death from serum hepatitis is at times complex and difficult to evaluate; it is not always possible to be certain that serum hepatitis was the only cause of death or even the major cause. Moreover, some patients die months to years later from chronic liver disease in which serum hepatitis may have been the inciting cause. In a few patients, serum hepatitis may have been superimposed upon a pre-existing form of chronic hepatitis or cirrhosis. The data shown in Table 6 represent the mortality experience from serum hepatitis at the University of Chicago Clinics. The total of 189 patients were accumulated over a period of eleven and one-half years, instead of the ten and one-half years referred to in other facets of this report.

II. The Use of the Sodium Salt of Monochloroacetic Acid as an Antibacterial Agent in Plasma and Whole Blood

Viral hepatitis is not the only contamination risk in transfusions of blood or plasma. Bacterial contamination, especially when the organisms are of the *Pseudomonas* and coliform groups, has been known to grow luxuriantly in citrate blood maintained at refrigeration temperatures for two to three weeks' time; several deaths from such accidents have been reported following transfusion.

A much greater risk from bacterial contamination exists when pooled plasma is allowed to stand for six months at 31.6° C. to minimize the risk of transmitting serum hepatitis. These conditions offer the maximal opportunity for luxuriant bacterial growth. Although we have been able successfully to avoid this problem at th University of Chicago Blood Bank by the use of scrupulous aseptic precautions checked by appropriate bacterial culture technics, it has been necessary to discard about 20 per cent of plasma pools in recent years. Thus, aside from the more serious consideration of the patient's welfare, the losses of plasma pools from bacterial contamination represent an ill-afforded extravagance of a product not readily available.

In consequence, about three years ago a study of this problem was instituted in this laboratory to find an antibacterial agent which could be safely added to whole blood as well as to plasma without distorting the therapeutic value of either transfusion fluid. It seemed important also that the antibacterial agent should retain its activity for indefinite periods of time, lest an isolated unit of plasma subsequently become contaminated. Since the possibility exists that such an agent might subsequently be shown to possess also a limited antiviral effect (hopefully virucidal), it is appropriate to summarize some of these observations at this time.

One method to achieve bacterial sterilization of plasma is the use of heat at 60° C. for several hours. Another is the addition of formaldehyde in 1 : 5,000 concentration. Both denature the proteins of plasma to an undesirable extent.[1, 8] The halogenated acetates were shown by Jensen [3] to protect against heat coagulation of plasma, an observation which encouraged us to test the effectiveness of these agents when added in nontoxic levels to plasma prior to heat-

TABLE 7. *Bacterial Growth After Inoculation of Human Plasma at 23°C*

Organism	Hours				Days	
	0	12	24	48	19	60
Staph. aureus						
1. Without ClAc̄ Rx*	10^8	10^9	10^9	10^9	10^8	10^7
2. With ClAc̄ Rx	10^9	0	0	0	0	0
Proteus mirabilis						
1. Without ClAc̄ Rx	10^7	10^8	10^8	10^8	10^8	10^6
2. With ClAc̄ Rx	10^6	0	0	0	0	0
Escherichia coli						
1. Without ClAc̄ Rx	10^8	10^8	10^9	10^9	10^7	10^6
2. With ClAc̄ Rx	10^8	0	0	0	0	0
Pseudomonas aerug.						
1. Without ClAc̄ Rx	10^8	10^8	10^9	10^9	10^8	10^7
2. With ClAc̄ Rx	10^8	0	0	0	0	0
Clostridium						
A. *Tetani*						
1. Without ClAc̄ Rx	10^7	10^6	10^4	10^5	10^4	10^3
2. With ClAc̄ Rx	10^7	0	0	0	0	0
B. *Perfringens*						
1. Without ClAc̄ Rx	10^5	10^4	10^3	10^2	10^2	10^2
2. With ClAc̄ Rx	10^5	0	0	0	0	0

* ClAc̄ = 0.02 molar chloroacetate.

To prepare 0.02 molar monochlorosodiumacetate, 28.35 grams of monochloroacetic acid (crystalline—Eastman Kodak) dissolved in 300 ml. of water, yielding a 1-molar solution. Twelve grams of sodium hydroxide as pellets are dissolved in this solution to bring its pH to approximately 7.7. Twelve ml. of molar acetate is added to 300 ml. of pooled plasma, giving a final concentration of approximately 0.02 moles of acetate per liter.

ing. Both bromoacetate and iodoacetate proved too toxic for clinical use. The monochloroacetate in concentrations of 0.02 or 0.04 moles per liter of plasma was nontoxic within the limits that volumes of plasma might be expected to be used acutely in the treatment of hypovolemic shock, or when given daily for nutritional purposes. However, chloroacetate afforded less protection against protein denaturation by heat and formaldehyde than did either the brom or the iodoacetate. Fluoroacetate, because of its interference with citrate metabolism, was not tested.

On highly speculative grounds, the postulate was made that monochloroacetate might itself have a potent antibacterial action. If so, the use of heat and formaldehyde for plasma sterilization could then be abandoned. Fortunately, monochloroacetate in concentrations of 0.02 M or 0.04 M per liter added to heavily contaminated human plasma displayed a surprisingly high degree of antibacterial activity. Some of the pathogens tested are shown in Table 7.

When the appropriate culture medium for a particular organism was liquid in form, one part of the culture broth was added to nine parts of acetate-treated plasma. In each instance, the culture and its organisms were not added until bacterial growth was maximal; thus, bacterial contamination was unusually heavy for those samples of plasma tested. Subsequent

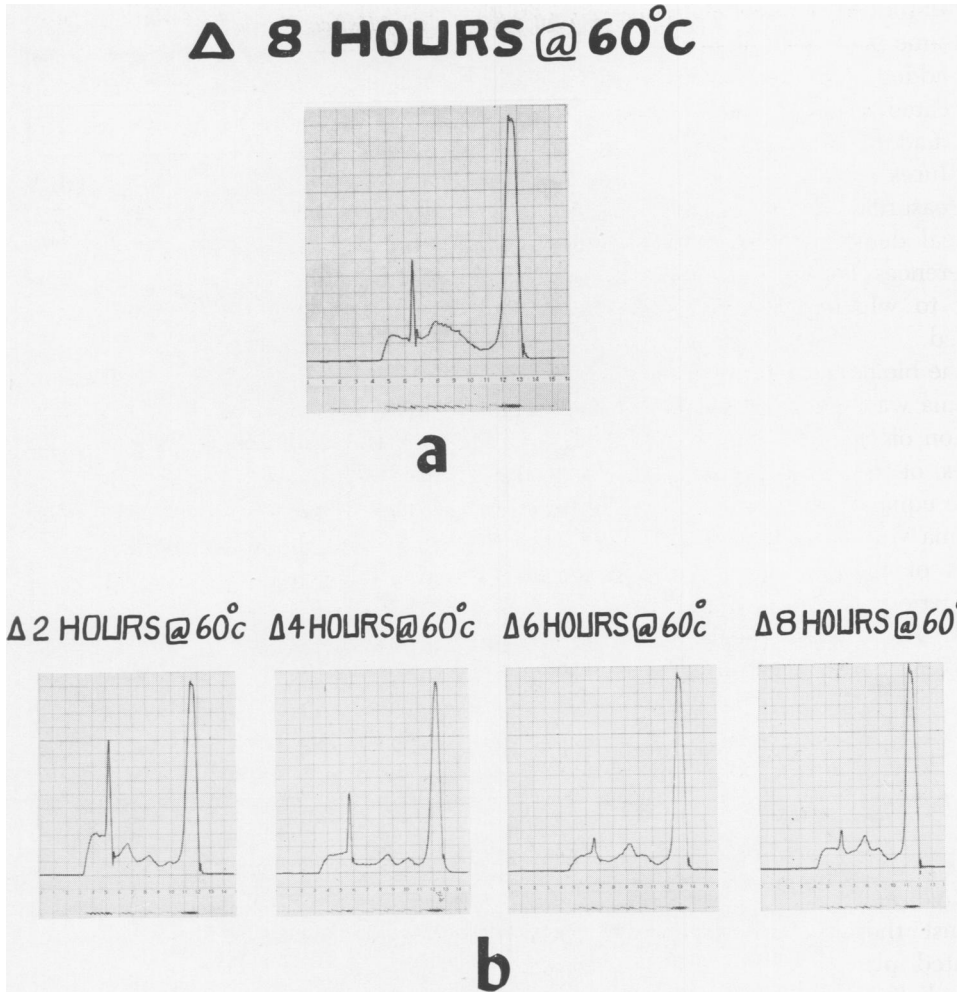

FIG. 1. Showing electrophoretic patterns of heated normal plasma above. The lower 4 patterns represent changes in electrophoretic patterns in plasma containing 0.02 molar chloroacetate after heating 2, 4, 6, and 8 hours respectively.

to inoculation, aliquots of contaminated plasma were held at 3° C., 23° C., and 37° C. The results shown in Table 7 are those obtained from contaminated acid-treated plasma held at 23° C.

Fortunately, monochloroacetate in the 0.02 M final concentration maintains its antibacterial action for at least seven months, despite frequent intentional bacterial contaminations introduced during this protracted period of time. This observation suggests that a pool or unit of plasma, whether originally contaminated or not, should maintain its bacterial sterility in spite of subsequent breaking of its seal which would permit bacterial contamination. We have not yet examined for the possibility that bacterial resistance to monochloroacetate may develop.

Paper electrophoresis (Spinco) was performed upon plasma containing monochloroacetate. The products examined were aged for varying periods of time with and without the presence of acetate. The electrophoretic results obtained disclose little if any change which did not also occur in

the aliquot of citrated plasma derived from the same pool when monochloroacetate was not added, provided both the treated and untreated samples were of the same age and had been maintained at similar temperatures (Fig. 1).

Measurements of plasma viscosity and optical density disclosed no demonstrable differences between ordinary plasma and that to which 0.02 or 0.04 M had been added.

The biologic tolerance for acetate-treated plasma was evaluated by the daily admnistration of canine plasma to dogs. The volumes of treated plasma given each day were equal to 50 per cent of the estimated plasma volume of the animal. These quantities of plasma were administered daily for periods of time ranging from 28 to 42 days. The animals were maintained on a nonprotein diet containing adequate minerals and vitamins throughout their test periods. The only source of protein available was that contained in the infused plasma. Both the volume of plasma and the total allowed calories were determined on the basis of body weight. To compare the relative merits of acetate-treated plasma against those of an aliquot of ordinary citrated plasma derived from a common pool, half of the animals received plasma containing 0.02 M of monochloroacetate per liter and the others, serving as controls, received citrated plasma without acetate.

The results of plasma infusions in both groups were identical throughout the entire study period. The animals remained afebrile, maintained their body weight, and were sustained in positive nitrogen balance. Other studies included erythrocyte, leukocyte, and thrombocyte counts on arterial blood and blood smears; hemoglobin determinations, bone marrow biopsy, prothrombin and whole blood clotting time, alkaline phosphatase and acid phosphatase activities, serum bilirubin, nonprotein nitrogen, and electrophoretic determinations. The results from these tests also disclosed no detectable difference between those animals receiving ordinary citrated plasma and those whose plasma also contained monochloroacetate. The same tests were continued following the completion of the infusion periods and no latent abnormalities thus far have been detected.

Biopsies performed on lung, liver, spleen, gut, adrenal glands, and the kidneys at the end of the infusion period in each animal disclosed no histologic abnormality.

Because we observed no evidence of toxicity from acetate-treated plasma in our fairly numerous experimental studies, it seemed appropriate to explore the therapeutic potential of human plasma to which monochloroacetate had been added to give a final concentration of 0.02 M or 0.04 M per liter of plasma. In each instance, the pooled plasma used had been aged for 10 weeks at 38° C. to minimize the risk of transmitting serum hepatitis prior to the addition of acetate. Thereafter the product was administered within a few days, 550 ml. to the unit.

To date, eleven debilitated patients suffering from carcinomatosis have received from 3 to 8 units of this plasma which they tolerated without detectable reactions or latent ill effects. Their general responses were those of improvement to the extent this was possible in patients suffering from a fatal illness. Laboratory studies, save for nitrogen balance and organ biopsies, were similar to those performed under the aforementioned experimental conditions. No abnormalities were detected. Two patients subsequently have died of their malignant disease; histologic examination of their tissues were normal, except for widespread carcinomatosis.

The possibility that acetate-treated plasma might prove to be allergenic cannot be answered. However, in two patients the administrations of plasma were separated by two- and four-week intervals with-

out evidence of untoward reactions of any kind.

A series of *in vitro* studies, similar to those of plasma, were carried out on whole blood. The antibacterial action of monochloroacetate was just as effective for blood as was observed in plasma. The organisms tested were the same as those added to plasma; the intensity of concentration of the bacterial culture added to blood was comparable to that employed in the studies on plasma.

Other studies on blood included the comparisons of acetate-treated blood with that for untreated aliquots from the same sample of citrated human blood. Among the qualities tested were red-cell fragility, their oxygen-carrying capacity, the life span of chromium-tagged red cells, hemoglobin diffusion into plasma when treated and untreated blood were allowed to age for 22 days at 3° C., spectrographic analyses for detection of abnormal hemoglobin pigment, and the concentrations of potassium in the supernatant plasma of the acetate-treated and the normal citrated samples derived from the same unit of blood. In each instance, no discernible difference was observed in the results obtained. Stated otherwise, these studies disclosed no harmful effects to whole blood when monochloroacetate had been added. Further studies are necessary to comment upon the toxicity following the transfusion of blood containing monochloroacetate, though in the limited extent to which these studies have been carried out, no toxic manifestations have been observed. A detailed report of these studies will be presented elsewhere.

Discussion

The serious problem of hepatitis from whole blood transfusions goes unacknowledged largely because there is no means whereby this complication can be anticipated, prevented, or treated. Further, its magnitude is partially obscured by the difficulty in tracing patients after transfusions. Thus it is necessary to accept serum hepatitis as a complication of whole blood, a calculated risk to be considered whenever blood is to be transfused. The magnitude of this risk, however, has not been generally appreciated.

The mortality in our patients transfused with blood without plasma was approximately one death from serum hepatitis for every 500 patients receiving transfusions of whole blood, a transfusion mortality from this cause alone which approximates that of appendectomy for nonperforative acute appendicitis. The over-all mortality from serum hepatitis in our series was 13.9 per cent. As pointed out earlier, the attack rate for hepatitis from blood transfusions in our patients may be higher than those experienced by others who need not rely so heavily upon the use of professional donors.

Risks, however, must be weighed against gains in any form of therapy. Thus, there is no question that blood replacement is imperative to the successful treatment of patients in shock due to losses of blood or plasma. Moreover, there is substantial clinical evidence to support the thesis that most patients recover more rapidly if normal blood volume is maintained. Further, the incidence of fatal postoperative pulmonary embolism and of acute renal failure appears to be greater in patients in whom shock occurs and is inadequately treated during surgical operations or following trauma. To withhold blood when clearly needed simply for the fear of transmitting serum hepatitis cannot possibly be justified.

However, the question should be asked: How important is a single 500-ml. unit of blood to the life of an adult patient if this is all that was necessary to restore his circulatory status? Would not another fluid or drug have served almost as well? Unfortunately, one does not always know that only one transfusion is needed; hence it is often better to start a transfusion rather

than to allow the patient's condition to deteriorate further. Thirty-six per cent of all patients transfused in this series received only one unit of blood; since the value of the single transfusion has been questioned in this institution for many years, it is our hope that this 36 per cent represents a smaller percentage of patients receiving single transfusions than may previously have obtained. Most transfusions given are probably necessary at the time they are given, but it also seems clear that some were given which may not have been required, at least in retrospect.

The potent antibacterial action of 0.02 M or 0.04 M monochloroacetate in blood or plasma and its low degree of toxicity, appears to warrant further exploration in both blood and plasma with the view to its possible routine use in the preparation of plasma transfusions as a possible means for the prevention of bacterial growth, should contamination occur unwittingly in the course of its preparation. If the observations herein reported be confirmed by others, the use of monochloroacetate may simplify many of the more cumbersome steps in plasma manufacture, especially those which concern its continuing bacterial sterility. Though the antibacterial action of monochloroacetate proved potent, the reasons which led us to postulate its use in this respect is open to serious question.

Further studies on toxicity of blood transfusions containing monochloroacetate are necessary before attempting a study on the use of this agent as an additive for clinical use in blood transfusions. The need for such an agent is self-evident and if proven effective and safe, its use would permit the return of unused blood to the blood bank for reissue, thereby conserving blood, especially that taken to the operating room as a precautionary measure in anticipation of a need which does not materialize. Present losses of blood incurred in those institutions performing surgery requiring the use of some form of extracorporeal circulation, have been especially distressing. A method which allowed the recovery and reissue of units of blood held at 37° C. for several hours in case of need during the course of open-heart surgery, would relieve a pressing problem in blood supply.

Several years ago Dr. Charles B. Huggins suggested to one of us (J.G.A.) the possibility that bromoacetate or iodoacetate might possess virocidal activity. At that time neither of us was aware of the antibacterial activity of monochloroacetate; we do not yet know that this agent has antiviral activity, though we are actively engaged in such a study at this time.

Summary

1. Thirty-six established cases of serum hepatitis occurred among a randomized selected series of 2,388 patients, 1,894 of whom survived at least six months after their last blood transfusion; eight other patients developed jaundice in which the diagnosis of serum hepatitis was held in question. An "average" of 3 units of blood was given each of the 2,388 patients in the series. Plasma or other icterogenic derivatives of blood had not been given. Five of the 36 patients died; two of the eight patients in which the diagnosis was questionable died.

a. Blood donor sources in this series were of two classifications: family donors (friends and relatives of the patient) and professional donors. The incidence of serum hepatitis with jaundice from professional sources was seven to ten times that experienced when all blood given was from family donors. For those patients who developed serum hepatitis when only one unit of blood was given, the attack rate was 0.3 per cent, whereas that of the single unit transfusions was 3.2 per cent when the unit was from a professional donor.

b. A total of 189 cases of serum hepatitis are known to have developed among the

total of 11,382 patients tallied as receiving blood only. These figures include the 2,388 patients in the randomized selection. Since only 21 per cent of 2,388 patients of the total patients receiving blood without plasma were fully followed until death or for at least six months after their last transfusion episode, the 145 cases which were known to have occurred among the nonstudy group, an additional 8,993 patients, it is probable that more cases of serum hepatitis could be detected in the nonstudied group were this group to be reviewed in its entirety. The total of 189 cases includes known cases developing through April 1, 1958, a period of time which was 15 months longer than the ten and a half years covered in the randomized sample.

c. The mortality rate for serum hepatitis in the 36 patients in whom this diagnosis was established among 1,894 patients receiving blood who survived six months, was 13.9 per cent.

2. Data reported on the antibacterial (bactericidal) activity of the sodium salt of monochloroacetic acid when added as a 1-molar solution to citrated blood or plasma so that its final concentration was 0.02 M or 0.04 M per liter of plasma or blood proved successful in eradicating heavy bacterial contamination with human pathogens which included vegetative and non-vegetative forms as well as the tuberculosis bacillus.

a. Surprisingly, plasma to which this acetate had been added displayed little if any detectable change in its electrophoretic pattern, viscosity or optical density.

b. Acetate-treated plasma was transfused to dogs in daily volumes equal to 50 per cent of the animal's estimated plasma volume for periods of 28 to 42 days. No abnormal physiologic reactions or pathologic effects were observed. In no way was the animal's response to transfusions of acetate-treated plasma different from that of ordinary citrated plasma without the monochloroacetate additive. To date, 11 debilitated patients with advanced carcinomatosis have received a total of 53 units (550 ml. each) of pooled plasma containing 0.02 M or 0.04 M of acetate; all were improved clinically to the extent expected from the administration of similar volumes of ordinary plasma.

c. Citrated whole blood to which monochloroacetate had been added (human and canine) gave no evidence that the life span of red cells was shortened, that the oxygen-carrying capacity of erythrocytes was altered, that red-cell fragility was increased, or that abnormal hemoglobin pigments developed. There was no evidence to suggest that citrated human blood containing this halogenated acetate was different from aliquots of the same units of blood serving as controls for the 22-day period covered by these *in vitro* studies; both the test and the control aliquots were maintained at 3° C. Although animals have been transfused freely with homologous canine blood containing 0.02 M or 0.04 M concentration of the acetate per liter, no deleterious effects have been noted, but data on the tolerance of blood transfusions are insufficient at this time to warrant comment upon the possible use of this additive to human transfusions.

d. The mode of action of this antibacterial agent has not yet been established.

e. We do not yet know if this agent has an antiviral propensity.

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Discussion

Dr. Paul Hoxworth: Mr. Chairman, Members and Guests: There are thousands of cases of serum hepatitis produced by blood transfusion each year. It is a serious disease, not only from the standpoint of the mortality which Dr. Allen expressed, but there is a serious incidence of chronic hepatitis and cirrhosis which often follows recovery from the acute phase.

Dr. Allen's paper is of importance to surgeons because they are the big users of blood, and as such should consider the risk and reduce the transmission as much as they can until a practicable method for eliminating viral activity is discovered.

(Slide) Dr. Allen's 44 cases of hepatitis from 5,000-odd units of whole blood, an incidence of infectivity of .728, means that one unit in 127 transmitted active disease to the recipient. This should cause the perfusionists to think.

Our experience of 19 cases from 7,135 units, an incidence of infectivity of .259%, means that one unit in each 386 transmitted a disease. When projected, the difference in these figures would represent the difference between 40,000 and 12,000 cases of serum hepatitis each year in the United States.

(Slide) The method of analysis which Dr. Allen has chosen is the attack rate obtained by dividing the cases of hepatitis by the number of recipients. Allen's attack rate is 2.3% and ours is 0.56%. There were no paid donors in our study, and the attack rate in Allen's group with only family donors is strikingly similar to ours. Considering that Dr. Allen's exposure was 3 units per patient, and ours was 2.4, it is almost identical.

In the two series the screening methods are identical and the geographical areas are contiguous. The higher incidence rate, as Dr. Allen has shown, is apparently due to the use of the paid donor. The purchase of blood at low rates attracts many alcoholics or other unfortunates who return every eight or ten weeks and who know that they will not get the money if they answer "Yes" to questions not only about jaundice but malaria and other infectious diseases.

Blood banks should pay donors only after exhausting other methods of recruitment, and only then from controlled groups whose medical history is known before they volunteer.

The evidence presented here today is further proof that there is no substitute for a physician's recruitment of donors among the families and friends of the patient, which is the system used exclusively in Cincinnati. This eliminates the costly and unwieldy methods which incorporate donor clubs, advance deposit insurance plans, paid publicity directors, expensive literature, and mobile units, as well as some of the hepatitis.

One more point. Another way for reducing disease transmission in transfusions is by avoiding unnecessary transfusions. Just as in Allen's series, in our institution thirty to forty per cent of the total volume of transfusions is now being given in single-point transfusions, most of which are probably unnecessary.

Dr. Frank B. Berry: I really didn't expect to discuss, but this whole problem is of enormous importance to all of us. I want to compliment both Dr. Allen and Dr. Hoxworth on their perseverance in carrying this work forward and bring-