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STUDIES ON THE SLOW CONTRACTION OF SMOOTH MUSCLE PRODUCED BY HUMAN PLASMA

BY

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The slow contraction of smooth muscle produced by human plasma becomes less potent after storage of the plasma for six months at room temperature and after processing of the plasma with kaolin. These observations fall into line with the fact that 40% of the initial smooth-muscle-contracting activity of the plasma can be accounted for in the deposit which is formed during the six-months period of storage, and also with the fact that the recovery of G acid from human plasma treated with kaolin is about 50% of the yield which is obtained from the same plasma before kaolin treatment.

Plasma is known to produce a quick contraction of the isolated guinea-pig ileum which is due to the presence of histamine or 5-hydroxytryptamine. It has recently been shown that after dialysis, which removes these agents, certain plasmas produce a delayed (at least 15 sec), slowly developing contraction of the ileum which is probably due to the formation of kallidin (or bradykinin) due to activation, by dilution, of an enzyme in plasma which releases it from a precursor which is also present (Schachter, 1956).

When larger volumes of human plasma are tested, evidence of another agent producing a delayed contraction appears. In this case there is a delay of only 4 to 10 sec after the addition of plasma to the isolated ileum, which then shortens fairly quickly, the response reaching a maximum within 30 sec. Recently, Gabr (1956) described the isolation from human plasma of a fatty acid which has a similar effect on the guinea-pig intestine. The present paper deals with the slow contraction produced by pooled plasma treated with kaolin (Maizels, 1944) and by pooled plasma stored at room temperature over a period of six months (Allen, Sykes, Enerson, Moulder, Elghammer, Grossman, McKeen & Galluzzi, 1950). The effect of pooled plasma on this contraction has also been studied.

In the method in which plasma is treated with kaolin, fibrinogen is removed from citrated plasma by adsorption, so that a fibrinogen-free plasma results which will not clot on filtration. Transfusion with this material has been particularly free from reactions, which suggests that kaolin may remove toxic substances.

According to Allen *et al.* (1950), pooled plasma stored in the liquid state without preservatives at room temperature for six months or longer before use produced no cases of homologous serum jaundice on transfusion when this was the only product administered. The virus of homologous serum jaundice in pooled plasma, which

has been stored in a liquid state at room temperature for six months, becomes attenuated and seldom if ever transmits the disease (Allen, 1952). Plasma stored in this manner for 18 to 24 months appears as effective for nutritive purposes as when stored for six months or less. These authors reported an over-all reaction to plasma stored at room temperature which was less than that to whole blood transfusion. The decreased reaction rate after the use of kaolin-treated plasma and room-temperature-stored plasma prompted the study of their effect on smooth muscle.

METHODS

Preparation of citrated plasma for storage at room temperature. Plasma was prepared from outdated blood (2 weeks or older). It was separated from the sedimented red cell mass and then stored at room temperature. Each blood transfusion was drawn in anticoagulant solution containing 2.2% trisodium citrate, 0.8% citric acid and 2.45% dextrose in pyrogen-free water (15 ml. solution for each 100 ml. blood). The blood was stored at 4° C to 6° C until transfused as such or converted to plasma, and was generally pooled with plasma from 10 donors. The plasma was then siphoned into standard M.R.C. plasma transfusion bottles under aseptic conditions. This plasma was stored at room temperature without preservatives. The plasma was prepared in a laboratory located in the same building as the donor centre. The temperature of the room in which the plasma was stored was recorded daily. Temperatures recorded during the period of storage varied from 14° C to 34° C. During room-temperature storage, monthly samples were taken for sterility control, chemical analysis and assay on the isolated guinea-pig ileum.

Isolation of G acid from the deposit formed in plasma stored at room temperature. Sterile human plasma slowly forms a precipitate as it ages, about 250 to 300 mg of precipitate per 100 ml. plasma being formed in six months. Chemically the deposit is similar in composition to the deposit which is formed in Seitz-filtered serum (Francis, Harrison & Picken, 1944). It consists of approximately the same amounts of calcium soaps, cholesterol and calcium phosphate. The percentage of nitrogen (13%) is, however, much higher than in the case of serum (1%), a fact which may be attributed to the clotting components which are readily denatured during storage at room temperature.

The precipitated material from twenty 500-ml. M.R.C. plasma bottles, each of an average age of 6 months, was collected by centrifugation and washed three times with distilled water, the precipitate being compacted by centrifugation after each washing. The compacted material was then dried to constant weight in a vacuum desiccator, acidified with concentrated hydrochloric acid and the liberated fatty acids were extracted with ether. After the removal of ether under reduced pressure, the ether extract was neutralized with 0.01 N sodium hydroxide. The material obtained was then processed according to the method of isolation of G acid from human plasma (Gabr, 1956), as follows:

Step 1. Removal of material insoluble in acetone.
Step 2. Chromatography on alumina (Savory & Moore).
Step 3. Acetic acid precipitation.
Step 4. Ether extraction and crystallization.

The crystalline product (4 mg, m.p. 34° C) has been chemically and biologically identified with G acid.

Processing of citrated plasma with kaolin. Colloidal kaolin (Leather Trades Specification) was placed in a 2-litre plasma Winchester (200 g/2 litre of pooled plasma), 10 ml. of saline was then added, and the whole sterilized by autoclaving at 20 lb./sq. in. for 1 hr. The plasma was then transferred from the Winchester bottle to the kaolin, left at 2° C for a day, shaken again and then left for a further 5 days to settle. The plasma was then siphoned into standard M.R.C. plasma transfusion bottles under aseptic conditions. Samples of pooled plasma were drawn from each Winchester before and after mixing with kaolin, for sterility

control, chemical analysis and assay on the isolated guinea-pig ileum. The plasma bottles were then left to stand at room temperature for 6 months in a place protected from direct sunlight as in the previous experiments. During storage at room temperature, monthly samples were taken for sterility control, chemical analysis and assay on the isolated guinea-pig ileum.

Sterility control. The neck and cap of the M.R.C. plasma bottle were flamed, and a 1 ml. sample was inoculated into 5 ml. of molten agar (45° C to 48° C). The agar bottles were then sloped on a glass rod and left to solidify at room temperature for 3 days, then incubated for 4 days at 37° C. The tests were read and infected bottles were discarded.

During room-temperature storage of the plasma, the following 2 tests were carried out at monthly intervals. Under sterile conditions, 10 ml. of plasma was drawn from the M.R.C. bottle and inoculated in 50 ml. of each of the following two media: (1) broth and (2) brewer's. In both tests incubation was carried out for 3 days at room temperature and 4 days at 37° C. The tests were read and the infected bottles were discarded.

Biological assay. One M.R.C. plasma bottle siphoned from each pool (normal plasma and kaolin-treated plasma pools) was transferred to ampoules, each holding 10 ml. of the plasma and dried from the frozen state by "Edwards Model 3PSA Centrifugal Freeze-Dryer" on the same day when the plasmas were distributed into the M.R.C. bottles. The moisture content of the product was then reduced to less than 1% by secondary desiccation over phosphorus pentoxide under high vacuum. The ampoules were then filled with dry nitrogen and sealed.

At monthly intervals a liquid sample drawn from each pool and its duplicate dried sample from the same pool before room-temperature storage were assayed on the isolated guinea-pig ileum. The determinations on the isolated guinea-pig ileum were made using a chamber of 55 ml. capacity filled with oxygenated Tyrode solution, the standard of reference being the reconstituted dried specimen taken from the same plasma pool, on the first day of storage. Plasma in doses of 2 to 4 ml. added to the Tyrode solution in the isolated organ bath caused contraction of the guinea-pig ileum after a latent period varying from 4 to 10 sec, depending on the sensitivity of the preparation. After the plasma had been in contact with the preparation for 30 sec, the preparation was washed by allowing the test fluid to flood over the rim of the organ bath. The contraction persisted after washing, slowly returning to the base-line. This took from 1 to 2 min. It is important to note that freeze-drying had no significant effect on the slow contraction of the isolated guinea-pig ileum, produced by citrated plasma (Fig. 1). The composition of Tyrode solution used as the test fluid in grams per litre is as

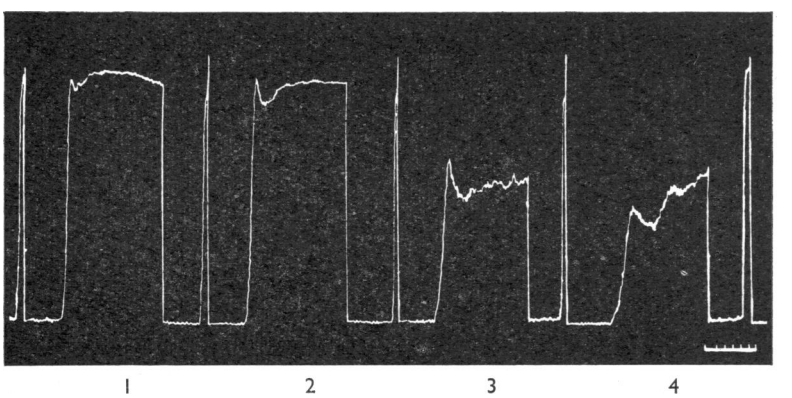

Fig. 1. The response of the isolated guinea-pig ileum to (1) 5 ml. liquid plasma, (2) 5 ml. reconstituted freeze-dried plasma, (3) 2 ml. reconstituted freeze-dried plasma, (4) 2 ml. liquid plasma, and to 0.2 μg acetylcholine (not numbered).

follows: 8.0 g sodium chloride, 0.2 g potassium chloride, 0.2 g calcium chloride, 0.01 g magnesium chloride, 0.05 g sodium dihydrogen phosphate, 1.0 g sodium bicarbonate and 1.0 g dextrose.

Chemical studies. Estimation of the plasma *p*H was made by the glass electrode technique, total plasma protein and non-protein nitrogen by micro-Kjeldahl.

RESULTS

Pooled plasma. Pooled plasma is generally less active on the isolated guinea-pig ileum than single donor plasma. This effect seems to depend on the size of the plasma pool; plasma pooled from a smaller number of samples was generally more active than that derived from a larger number. Thus, plasma pools derived

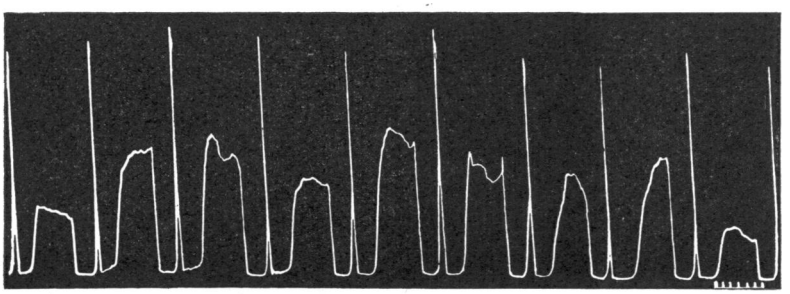

Fig. 2. The response of the isolated guinea-pig ileum to (1) 2 ml. pooled plasma, (2) 2 ml. plasma (blood bottle no. 896, blood group B), (3) 2 ml. plasma (bottle no. 61, blood group AB), (4) 2 ml. plasma (bottle no. 2342, blood group A), (5) 2 ml. plasma (bottle no. 2354, blood group A), (6) 2 ml. plasma (bottle no. 2357, blood group A), (7) 2 ml. plasma (bottle no. 2359, blood group A), (8) 2 ml. plasma (bottle no. 6102, blood group O), (9) 2 ml. pooled plasma, and to 0.2 μg acetylcholine (not numbered).

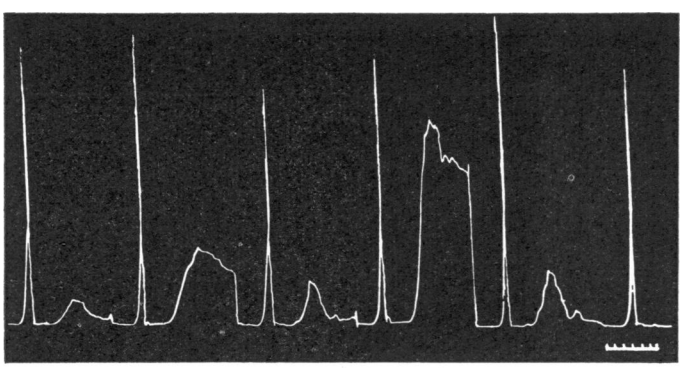

Fig. 3. The effect of the size of plasma pool on the slow contraction of the isolated guinea-pig ileum produced by pooled plasma. Response of the guinea-pig ileum to (1) 4 ml. pooled plasma (plasma pool derived from 10 blood bottles), (2) 4 ml. pooled plasma (plasma pool derived from 7 blood bottles), (3) 4 ml. pooled plasma (plasma pool derived from 10 blood bottles), (4) 4 ml. pooled plasma (plasma pool derived from 3 blood bottles), (5) 4 ml. pooled plasma (plasma pool derived from 10 blood bottles), and to 0.2 μg acetylcholine (not numbered).

from 10 donations of blood were found to be less active on the isolated ileum than plasma pools derived from 7 donations of blood. Again, the latter were less active than those derived from 3 blood donations. All of these plasmas were prepared from different group donors but always including a group B blood in each pooled sample. When the samples were derived from 10 or more donations of blood, the onset of muscle contraction was much slower, and the maximum contraction often declined within a few seconds. Fig. 2 shows the effect of pooling on the slow contraction of smooth muscle produced by plasma from a single donor. No relation could be found between the toxicity of pooled plasma for smooth muscle and the group types of the blood bottles from which the plasmas were separated. Fig. 3 shows the effect of the size of pool on the slow contraction of the isolated guinea-pig ileum produced by pooled plasma. It may be interesting to note that the activity of pooled plasma on smooth muscle decreased slightly after standing for one week at room temperature prior to freeze-drying.

Plasma stored at room temperature. The smooth-muscle-stimulating activity, as measured on the guinea-pig ileum, of liquid pooled plasma stored at room temperature for 6 months decreases. The decrease is gradual and varies from one batch

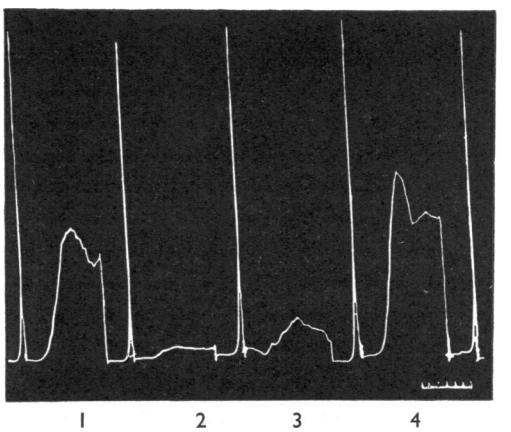

Fig. 4. The effect of room-temperature storage of plasma on the slow contraction of the isolated guinea-pig ileum produced by normal plasma and kaolin-treated plasma. Responses to (1) 3 ml. reconstituted dried kaolin-treated plasma (pool no. 241), (2) 3 ml. kaolin-treated liquid plasma stored at room temperature for six months (pool no. 241), (3) 3 ml. liquid plasma stored at room temperature for six months (pool no. 230), (4) 3 ml. reconstituted dried plasma (pool no. 230), and to 0.2 μg acetylcholine (not numbered).

to another. The contraction starts more slowly and relaxation takes much longer in spite of repeated washings of the organ bath with Tyrode solution. The plasma becomes more turbid and does not clot after the addition of calcium chloride. Fig. 4 shows the effect of storage on the slow contraction produced by normal liquid plasma on the isolated guinea-pig ileum.

Average results of 5 pools show that the *p*H of normal liquid plasma decreases from a normal value of 7.05 to 6.80 over the six-months period. The total protein

decreases from a normal value of 5.8% to 5%. This decrease in total protein is more marked in this series of experiments than in those experiments reported by Taylor, Lozner, Davidson, Tagnon & Newhouser (1944), in which glucose and merthiolate were added to liquid plasma for preservation. The non-protein nitrogen of liquid plasma does not show significant alteration during storage at room temperature (Table 1). Cultures for bacterial growth were uniformly negative.

TABLE 1

AVERAGE VALUES OF MEASUREMENTS MADE ON NORMAL LIQUID PLASMA STORED AT ROOM TEMPERATURE OVER A PERIOD OF SIX MONTHS (5 POOLS)

0=Baseline values which were also given by the freeze-dried duplicate specimens after reconstitution with distilled water

Period of storage (months)	*p*H±s.d	Total protein (g/100 ml.) ±s.d.	Non-protein nitrogen (mg/100 ml.) ±s.d.
0	7·05±0·17	5·8 ±0·17	21·7±1·4
1	7·05±0·32	5·55±0·17	22·7±1·3
2	7·00±0·1	5·05±0·45	21·4±3·9
3	6·95±0·1	4·8 ±0·15	21·7±0·4
4	7·00±0·24	4·9 ±0·1	23·4±2·0
5	6·9 ±0·1	5·1 ±0·25	22·4±2·0
6	6·8 ±0·16	5·0 ±0·3	21·9±2·4

Analysis of the deposit which is formed in liquid plasma during the six-months period of storage at room temperature showed that about 10% of the original smooth-muscle-stimulating activity of the plasma can be recovered from the deposit. Since the total recovery of this activity is about 25% of that of the original active material (Gabr, 1956), one may conclude that the deposit which is formed during storage at room temperature retains 40% of the original smooth-muscle-stimulating activity of the plasma. This conclusion may explain the marked decrease in the effect of liquid plasma on the isolated guinea-pig ileum after the six-months period of storage at room temperature (Gabr & Aly, 1959).

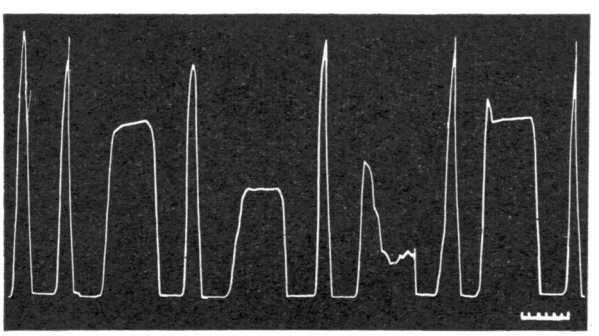

Fig. 5. The response of the isolated guinea-pig ileum to liquid plasma before and after processing with kaolin. Responses to (1) 4 ml. reconstituted freeze-dried plasma, (2) 4 ml. reconstituted freeze-dried plasma which had been treated with kaolin, (3) 4 ml. kaolin-treated plasma, kept at room temperature for one week, in the liquid state, (4) 4 ml. reconstituted freeze-dried plasma, and to 0.2 μg acetylcholine (not numbered). All specimens were taken from the same plasma pool.

Kaolin-treated plasma. Processing of pooled plasma with kaolin decreased its effect on the isolated guinea-pig ileum. The effect of kaolin was more marked at room temperature than at 2° C. Like normal plasma, kaolin-treated plasma became less active on the isolated guinea-pig ileum after storage for six months at room temperature. The final product was less active than normal plasma stored under the same conditions without preservatives. Kaolin-treated plasma is clearer than normal plasma, and develops no turbidity during storage in spite of the relatively big variation in room temperature.

Fig. 5 shows the response of the isolated guinea-pig ileum to liquid plasma before and after processing with kaolin. The effect of storage at room temperature on the slow contraction produced by kaolin-treated plasma is shown in Fig. 4.

Average results of 5 pools showed that processing with kaolin does not significantly affect the *p*H or the non-protein nitrogen of the plasma. There was, however, a marked drop in total protein (from a normal value of 6.2% to 4.9%). The *p*H of kaolin-treated plasma increased slightly from a normal value of 7.0 to 7.2 over the six-months period of storage at room temperature. The total protein decreased from an average value of 4.9% to 4.5%. The non-protein nitrogen of kaolin-treated plasma did not show significant alteration during the same period of storage (Table 2). Cultures for bacterial growth were uniformly negative.

TABLE 2

AVERAGE VALUES OF MEASUREMENTS MADE ON KAOLIN-TREATED LIQUID PLASMA STORED AT ROOM TEMPERATURE OVER A PERIOD OF SIX MONTHS (5 POOLS)

0=Baseline values which were also given by the freeze-dried duplicate specimens after reconstitution with distilled water

Period of storage (months)	*p*H±s.d.	Total protein (g/100 ml.) ±s.d.	Non-protein nitrogen (mg/100 ml.) ±s.d.
Before treatment	6·9 ±0·2	6·2±0·24	22·7±3·1
0	7·0 ±0·18	4·9±0·23	21·7±0·9
1	7·1 ±0·14	4·9±0·17	21·0±0·7
2	7·2 ±0·1	4·9±0·17	22·9±1·1
3	7·25±0·12	4·9±0·25	22·9±1·2
4	7·3 ±0·12	4·7±0·15	22·9±1·0
5	7·3 ±0·24	4·6±0·25	22·9±1·1
6	7·2 ±0·12	4·5±0·19	22·9±1·1

The deposit which is formed in kaolin-treated plasma during the six months of storage at room temperature is much similar in its chemical composition to the deposit which is formed in human serum under the same conditions of storage. The recovery of smooth-muscle-stimulating activity from this deposit by the method described is very small when compared to that of untreated plasma.

DISCUSSION

Some of the reactions which may occur following the administration of human plasma are characterized by violent pain in the back, vomiting and defaecation. These reactions have been attributed to certain pharmacologically active substances, the presence of which in serum and plasma has been demonstrated by Reid &

Bick (1942a & b). Lozner & Newhouser (1944) reported a decreased reaction rate upon prolonged storage of normal citrated plasma at room temperature. Clinical experience with the material stored at room temperature showed that younger plasma was followed by reactions more often than older plasma, a phenomenon which was attributed by the authors to the disappearance of some mildly toxic labile constituent. There is also some evidence that processing of the plasma with kaolin (Maizels, 1944) removes some of the pharmacologically active substances from the plasma, and this was borne out clinically by the fact that kaolin plasma seemed remarkably free from all reactions. Although a number of pharmacologically active substances have been isolated from or identified in plasma, the relationships between these pharmacologically active substances to the reactions described have never been proved clinically. The results cited in this paper show that storage at room temperature decreases the slow contraction of smooth muscle produced by citrated plasma. Processing of citrated plasma with kaolin produces a similar effect. This effect may arise from partial precipitation of the causal factors during storage at room temperature of the plasma in the first case and partial adsorption of the causal factors on kaolin in the second case. These conclusions are borne out biologically by the fact that 40% of the starting smooth-muscle-contracting activity of citrated plasma could be accounted for in the deposit which is formed during the six-months period of storage at room temperature in the first case, and by the fact that the recovery of G acid from kaolin plasma is about 50% of the yield obtained from the same plasma before kaolin treatment in the second case. Attempts to recover G acid from the kaolin used in this method were unsuccessful.

The decrease in the total protein concentration of the plasma during storage at room temperature cannot account for the lower activity of the final product on the isolated guinea-pig ileum, since it was found that the response of this preparation to different plasmas was not parallel to their total protein content. The changes in *p*H and non-protein nitrogen of the plasma during storage at room temperature and after kaolin treatment were too slight to influence the response of the isolated guinea-pig ileum to the slow contracting principle of the plasma.

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