shows that the results were satisfactory, since the plasma inulin level became virtually constant after three to four hours. It is inferred that this is the minimum time limit for an inulin infusion for the purpose of measurement of inulin clearance by the urineless technique. Secondly, the exact rate of infusion of inulin must be known. Not only must the exact volume of inulin solution infused per minute be known, but also the exact concentration. The latter is not known beforehand, since the mere heating of the solution to dissolve inulin results in some breakdown to fructose (Durbin and Rose, 1969). Therefore the infusate must be analysed for inulin and fructose separately and only the true inulin taken into account. This point is met in the technique described here by measuring the inulin with and without hydrolysis.

The agreement between clearances by the two techniques (with and without urine) appears at first sight to be rather poor (Fig. 2). A closer study, however, shows that there are two types of results, those where the two (or three) consecutive results from urine collections agreed well with each other and those in which they did not. In 10 studies the clearances calculated by the two methods differed by less than 12 ml./minute, and in this group the mean range of clearances for successive urinary periods was 12·4 ml./minute. In six studies the clearances calculated by the two methods differed by 23–42 ml./minute, and the mean range of clearances for successive urinary periods was 25·9 ml./minute. These results are separately indicated in Fig. 2, and it is quite obvious that the two methods correlated poorly in those cases where the urine collections were unreliable, a result that is not at all surprising for specimens obtained without catheterization. On the other hand, when urine collections were reliable, as shown by good agreement for uv/p in successive periods, these results correlated well with the urineless method. It should also be noted here that a large residual volume of urine would result in the urineless method giving a higher clearance value than the classical method, and this is well demonstrated in Fig. 2, where substantial disagreements between the two methods are always in this direction.

It is concluded that the urineless method of assessment of renal clearance devised by Earle and Berliner (1946) is satisfactory for inulin provided that an equilibration time of at least four hours is allowed for non-oedematous patients. This period of time is much longer than that used by Berger *et al.* (1948). An inulin infusion even of this length of time is scarcely even an inconvenience, however, for an inpatient if modern plastic intravenous catheters are used. This means that for non-oedematous individuals with good renal function an extremely simple method for measurement of inulin clearance can be used. It is only necessary to infuse the inulin at a known constant rate for four hours and measure the final plasma inulin level together with the concentration of inulin in the infusate. These measurements are readily and accurately carried out by the method described. The advantages of not having to collect urine do not require further mentioning. The advantage of using inulin is that, thanks to the extensive research of earlier workers (see Homer Smith, 1951), it seems that inulin clearance is a measure of glomerular filtration rate. Inulin is non-toxic, reasonably cheap, and it is not necessary to burden the patient with radioactive materials. It is suggested that the urineless method for measurement of glomerular filtration rate by means of inulin infusion should find widespread application for non-oedematous subjects.

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Preliminary Communications

Anti-Inflammatory Properties of Human Inflammatory Exudate

British Medical Journal, 1969, **2**, 93–96

Summary: An inflammatory exudate collected from a site of major surgery (partial gastrectomy) was found to possess definite anti-inflammatory properties when tested by the carrageenin oedema technique (a method widely adopted for the assessment of potential anti-rheumatic agents). Such anti-inflammatory properties could not be detected in the serum of normal healthy adults or in an abdominal ascites fluid. The active component in the exudate showed several properties in common with a similar anti-inflammatory substance present in inflammatory exudates of animal origin.

INTRODUCTION

More than a decade has passed since anti-inflammatory properties were first attributed to an inflammatory exudate (Rindani, 1956). The initial observation of Rindani has been confirmed several times with a number of inflammatory exudates of animal origin (DiPasquale and Girerd, 1961; DiPasquale, Girerd, Beach, and Steinetz, 1963; Robinson and Robson, 1964, 1966). Nevertheless, the presence of anti-inflammatory properties, by which is meant the ability to inhibit experimental inflammatory reactions, has not as yet been described for an inflammatory exudate of human origin.

The early studies of Rindani (1956) established that the inflammatory exudate formed in the granuloma pouch in rats by injection of an irritant substance was capable of inhibiting in recipient rats formalin-induced arthritis of the hind foot. These findings stimulated DiPasquale and Girerd (1961) and

DiPasquale *et al.* (1963), working in the United States, to investigate further the properties of granuloma pouch exudate collected from the rat. They also demonstrated the anti-inflammatory activity of this exudate by showing that when injected into rats it produced a decrease in the granulation tissue reaction around implanted cotton-wool pellets.

These results were confirmed by Robinson and Robson (1964), working independently in England. Inflammatory exudate formation was induced in this instance by implanting a polyester sponge beneath the skin of the rat. The inflammatory exudate squeezed from the sponge possessed the ability to reduce granulation tissue deposition around implanted cotton pellets, and in this respect was comparable to the inflammatory exudate of DiPasquale and his co-workers.

With regard to the nature of the active principle, both DiPasquale and his colleagues and Robinson and Robson (1966) showed that the inflammatory exudate collected from adrenalectomized rats, as well as from intact donor rats, possessed anti-inflammatory properties, and it was thus concluded that the active substance was not of adrenal origin. This conclusion was taken a step further by fluorimetric estimation of the 11-hydroxycorticosteroid content of the inflammatory exudates (DiPasquale *et al.*, 1963 ; Billingham, 1968), when it was shown that no significant amounts of steroids were present. The characterization of the active principle in the exudate obtained by polyester sponge implantation into rats has been pursued a little further. Robinson and Robson (1966) found that the active anti-inflammatory component was non-dialysable—that is, it behaved as a macromolecule. Continuing the investigations with another inflammatory model—the carrageenin-induced oedema of the rat hind foot—Billingham, Robinson, and Robson (1969) confirmed the non-dialysable nature of the active constituent. In addition it was shown that the anti-inflammatory substance was thermolabile, was destroyed by enzyme digestion, and behaved as a protein when subjected to the protein fractionation techniques of Sephadex gel filtration and cellulose ion-exchange chromatography. It was concluded from the above findings that the active constituent was probably protein in nature.

Our knowledge concerning the anti-inflammatory properties of inflammatory exudates of animal origin has thus progressed to a fairly definitive state. In the present paper data are presented which show that anti-inflammatory activity is present in an inflammatory exudate collected from man.

The most practical source of human inflammatory exudate was found to be that accumulating at sites of major surgery, and one type of surgical procedure—namely, gastrectomy—was chosen for this purpose. This choice was made as a result of the observation of Werner and Odenthal (1967) that the inflammatory lesion caused by partial gastrectomy was perhaps the best surgical model of the acute phase reaction, in that its time-course was quite constant from patient to patient.

Methods

Collection of Inflammatory Exudate

The inflammatory exudate, formed at the site of partial gastrectomy, was collected as the fluid escaped from peritoneal drainage. This fluid was collected either on dressings or in special drainage bottles. After removal from the patient the dressings or bottles were placed in sterile polyethylene bags and put in the deep freeze.

Exudate was eluted from the dressings by steeping in 100 ml. of 0.9% saline for two hours at 4° C., after which time the dressings were squeezed to remove the exudate. Cells and other debris were removed by centrifugation at 2,500 r.p.m. for 30 minutes at 4° C. Exudate from bottles was simply centrifuged. Any bacterial contamination of the exudate was removed by Millipore filtration. The exudate was passed through filters of progressively decreasing pore size to 0.22 mμ, after which the exudate was regarded as sterile, though this was not checked bacteriologically.

The resultant fluid after Millipore filtration was force dialysed to remove material of small molecular weight and to concentrate to a small volume. The exudate was then freeze-dried and stored at −20° C. until assayed for anti-inflammatory activity.

The anti-inflammatory activity of the human inflammatory exudates was determined by using the technique of carrageenin-induced oedema of the rat hind foot as described by Winter, Risley, and Nuss (1962). Intact male albino Wistar rats weighing between 140 and 180 g. were used for the anti-inflammatory assay. In all experiments the rats were randomly divided into groups of five, so that each group had the same mean body weight. Oedema formation was induced in the paw by injection of 0.1 ml. of a 1% solution of carrageenin (Viscarin, Marine Colloids Inc.) in sterile 0.9% saline into the plantar aponeurosis of the right hind foot. The injections were given under light ether anaesthesia, and at this time the substances to be tested for anti-inflammatory activity were injected subcutaneously in the neck region.

The volume of the foot was measured plethysmographically by the method of Robinson and Robson (1966) except that the animals were not anaesthetized. Measurements of paw volume were made before the injection of carrageenin, and 2½, 5, and 24 hours afterwards, and the mean increase in paw volume over the uninjected paw volume was calculated.

Sephadex Gel Filtration

This technique provided a means of separating the proteins of the inflammatory exudate on the basis of their molecular size. The precise technique used was that recommended by the manufacturers of Sephadex (Pharmacia). Sephadex G 150 gel was used in a column 100 cm. long and 5 cm. in diameter. The column was eluted at a rate of 65 ml./hour with 0.5M sodium chloride solution, and the protein content of the eluted fluid was measured as the percentage transmission at a wavelength of 253 mμ.

Results

To date, inflammatory exudates have been collected from five patients who underwent partial gastrectomy, and these have been tested for anti-inflammatory activity by the carrageenin paw-oedema technique. The inflammatory exudates from each patient were collected daily in order to study the appearance of anti-inflammatory activity in the exudate. In

Table I.—*Effect of Inflammatory Exudate Collected Daily from Partial Gastrectomy Patients on Carrageenin-induced Oedema of the Rat Hind Paw*

Case No.	Sex	Anti-inflammatory Effect of 100 mg. of Inflammatory Exudate per Rat shown as Percentage Inhibition of Paw Swelling seen 5 Hours after Carrageenin Injection					
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	M	—	—	—	47 ($P < 0.005$)	53 ($P < 0.005$)	64 ($P < 0.005$)
2	M	26 (N.S.)	39 ($P < 0.02$)	51 ($P < 0.005$)	64 ($P < 0.001$)	46 ($P < 0.005$)	—
3	M	11 (N.S.)	28 ($P < 0.025$)	—	—	—	—
4	F	—	24 ($P < 0.02$)	44 ($P \ll 0.001$)	50 ($\ll 0.001$)	—	—
5	M	—	25 ($P < 0.05$)	38 ($P < 0.005$)	53 ($P < 0.001$)	47 ($P < 0.005$)	—
Mean		18	29	44	53	49	

order to do this it was necessary to collect at least 500 mg. of freeze-dried inflammatory exudate per day for the administration of 100 mg. of the exudate to each rat. On certain days, however, insufficient inflammatory exudate was obtained for testing in a group of five rats. A dose level of 100 mg. of freeze-dried, human inflammatory exudate per rat was chosen, because it had been shown that a dose of 100 mg. of freeze-dried inflammatory exudate of rat origin consistently produced a marked—that is, >50%—inhibition of carrageenin-induced oedema formation (Billingham, 1968).

Table I shows the anti-inflammatory activity of the inflammatory exudates obtained from the five partial gastrectomy patients who have so far been studied. The anti-inflammatory effect exerted by the exudates is depicted as the percentage inhibition of paw swelling—that is, oedema formation—when compared with saline-treated control animals. The statistical significance of the difference in paw swelling between the groups of animals receiving inflammatory exudate and those receiving saline is also given (P value).

From Table I it is evident that two days after the operation significant anti-inflammatory activity is present in all inflammatory exudates collected from partial gastrectomy patients. The inhibitions of oedema formation produced by the two inflammatory exudates collected on the first day after the operation were not statistically significant.

The increase in anti-inflammatory activity occurring between days 2 and 4 in Cases 2, 4, and 5 was found to be statistically significant. This indicated that the concentration of the anti-inflammatory component in the exudate had risen during this time, since the actual dose of exudate was the same for each day of its collection—that is, 100 mg. per rat.

As a comparison the effect of control human protein solutions—namely, human serum and ascites fluid—was determined on carrageenin-induced oedema formation. Serum from two unoperated, adult donors, at a dose level of 100 mg. per rat of the freeze-dried material, produced respectively a 7% and a 12% inhibition of oedema formation. A freeze-dried peritoneal ascites fluid, at a level of 100 mg. per rat, produced a 16% inhibition of oedema formation. Neither of these effects was statistically significant.

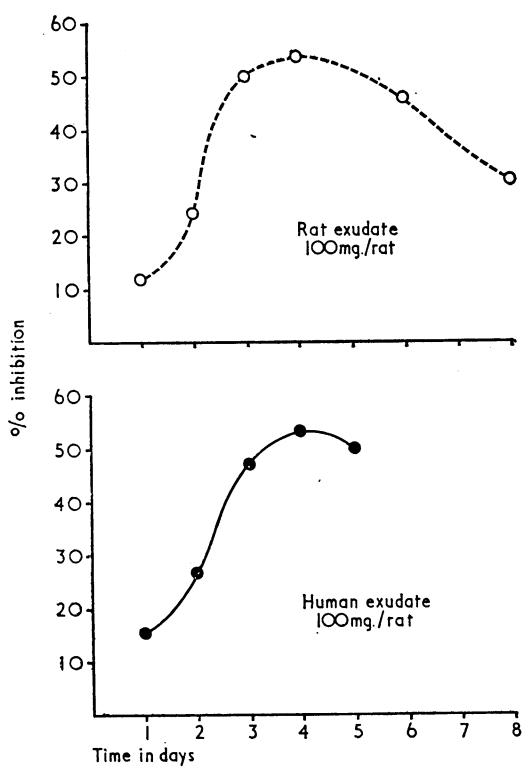

FIG. 1.—Effect of inflammatory exudate, collected at increasing time intervals, on carrageenin-induced oedema of the rat hind foot.

In Fig. 1 the mean values for the anti-inflammatory activity of the partial gastrectomy exudates on each day have been plotted against the day when exudate was collected (lower section of Fig. 1). For comparison Fig. 1 also shows the anti-inflammatory properties of rat inflammatory exudate, collected from polyester sponge implants on various days after implantation. The inflammatory exudate collected from the rat, like that of human origin, was forced dialysed and freeze dried before being administered to groups of five rats at a dose of 100 mg. per rat. The anti-inflammatory effect was determined as the percentage inhibition of oedema formation seen five hours after carrageenin injection, when compared with saline-treated control animals.

It is evident from Fig. 1 that in the inflammatory exudate collected from both the rat and the human the increase in anti-inflammatory activity seen with the passage of time is remarkably similar. Furthermore, the increase in anti-inflammatory activity occurring in the rat exudate between days 2 and 4 was statistically significant, like the increase in activity described above for the exudates from partial gastrectomy patients.

When subjected to Sephadex G 150 gel filtration the anti-inflammatory activity of human exudate separated in the same way as rat exudate (Billingham *et al.*, 1969). The elution diagram for the gel filtration of 500 mg. of freeze-dried human exudate—that is, sufficient exudate for one assay (100 mg. per rat)—is shown in Fig. 2. The dotted lines in this figure represent the poolings of separated proteins subsequently used in anti-inflammatory assay. The pooled proteins were force dialysed and freeze dried before they were assayed, and Table II shows the anti-inflammatory activity of these protein pools when tested in groups of five rats.

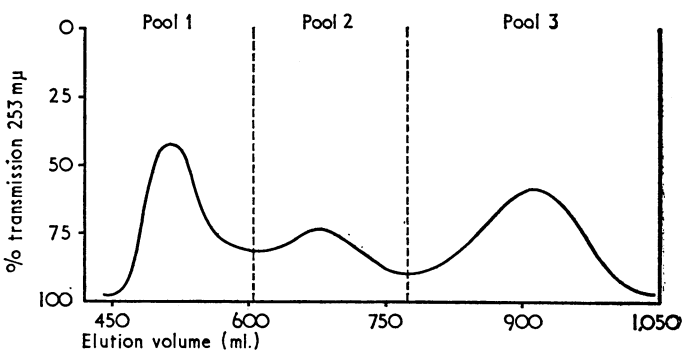

FIG. 2.—Elution diagram of the separation of 500 mg. of human inflammatory exudate by Sephadex G 150 gel filtration.

The proteins in pool 1 produced an insignificant inhibition of oedema formation, whereas both the other protein pools produced a highly significant inhibition of oedema.

TABLE II.—*Effect of Pooled Materials from Sephadex G 150 Gel Filtration of Human Exudate (Case 1) Given Subcutaneously on Carrageenin-induced Oedema of the Rat Hind Paw*

Treatment	Dose per Rat	Mean Increase in Paw Volume over Resting Volume ± S.E. at 5 Hours	Percentage Inhibition of Oedema, Compared with Control, and Significance (P)
Saline control ..		0·45 ± 0·03	
Pool 1	23 mg.	0·39 ± 0·04	13 (P > 0·2)
Pool 2	27 mg.	0·24 ± 0·03	48 (P < 0·001)
Pool 3	53 mg.	0·19 ± 0·03	58 (P ≪ 0·001)

DISCUSSION

There is now considerable information concerning the anti-inflammatory properties of inflammatory exudates of animal origin. It has been suggested that the ability of these inflam-

matory exudates to inhibit inflammatory reactions forms part of the body's normal mechanism for limiting and terminating acute inflammatory reactions (DiPasquale and Girerd, 1961 ; DiPasquale *et al.*, 1963 ; Robinson and Robson, 1964, 1966, 1967). Hence it was thought desirable to investigate whether a similar situation existed in man.

In this investigation an inflammatory exudate of human origin was shown to inhibit markedly an experimental inflammatory reaction (see Table I). This anti-inflammatory effect is not attributable simply to the injection of a foreign protein, since human serum and an ascites fluid were virtually devoid of anti-inflammatory activity. Another possible explanation is that the anti-inflammatory activity of partial gastrectomy exudate is due to the action of corticosteroids either present in the exudate or released in the recipient rats. Although at the moment we have no direct evidence to disprove this hypothesis, we do know that the exudate collected from adrenalectomized rats, and shown fluorimetrically to contain no corticosteroids (Billingham, 1968), markedly inhibits experimental inflammation in both intact and adrenalectomized recipient rats. It has not been possible to assess fluorimetrically the corticosteroid levels of the human exudates, since the haemolysis that is usually seen with these renders accurate estimation impossible. Nevertheless, the exudates collected immediately postoperatively, at a time when a higher level of steroid would be expected, have less anti-inflammatory activity than exudates collected subsequently. It seems more likely that the active component of human exudate, rather than being a steroid, is similar to the anti-inflammatory substance in animal exudates—that is, a protein. As evidence of this the similarities discussed below between the anti-inflammatory properties of inflammatory exudates of human and animal origin add some weight to the possibility of the active components being related.

The time of appearance of anti-inflammatory activity, in both types of inflammatory exudate, is remarkably similar in respect of both the rise in activity during the first few days and the time at which maximal activity is reached. With regard to its chemical nature the anti-inflammatory component of both inflammatory exudates is non-dialysable—that is, it behaves as a macromolecule. Both human inflammatory exudate and that from the rat fractionate in a similar fashion when subjected to Sephadex G 150 gel filtration. Furthermore, the anti-inflammatory activity appears in a similar position on the elution diagram—that is, in pools 2 and 3 (see Fig. 2 and Table II). It is possible, therefore, that the anti-inflammatory activity of human inflammatory exudate, as has been suggested for the rat, forms part of the body mechanisms involved in limiting and terminating acute inflammatory reactions.

Looking to the future, it is hoped to purify the anti-inflammatory substance of human origin at least to the stage reached with the anti-inflammatory substance obtained from the rat. The purification of the rat material has reached the point where 3 mg. of purified material produces a similar anti-inflammatory effect—that is, inhibition of oedema—to that produced by 100 mg. of the whole inflammatory exudate (Billingham *et al.*, 1969). On a weight-for-weight basis the purified rat material, though still far from pure, is as active as hydrocortisone.

It is hoped that ultimately the anti-inflammatory substance of human inflammatory exudate, when purified, may be of use clinically in the treatment of chronic inflammatory diseases.

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Medical Memoranda

Hypercalciuria in Sarcoidosis Treated with Inorganic Phosphates

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Hypercalciuria and hypercalcaemia are of particular importance, since they may lead to calculus formation or nephrocalcinosis and renal failure, hypercalcaemia being one of the most definite indications for corticosteroid therapy (Scadding, 1967).

The use of inorganic phosphate in the case described below was prompted by persistent hypercalciuria, which was only poorly controlled by large doses of prednisone.

CASE HISTORY

A man aged 30 was admitted to hospital on 31 August 1965 complaining of cough, dyspnoea, and loss of 1 st. (6·4 kg.) in weight over the previous two months. Clinical examination showed nothing abnormal.

Investigations.—Chest *x*-ray examination showed widespread bilateral nodulation with hilar gland enlargement. Tuberculin skin test (100 T.U.) was negative. Liver biopsy showed follicles of the sarcoid type. Serum calcium was 9·5 mg./100 ml. ; 24-hour urine calcium 810 and 1,000 mg. ; alkaline phosphatase 13·5 K.A. units ; total plasma proteins 7·5 g./100 ml. (albumin 4·1 g., globulin 3·4 g.). Electrophoresis showed slight increase in alpha$_2$-globulin. The gas transfer factor of the lung for carbon monoxide (T_{LCO}) at rest was 9·5 ml. CO/min./mm. Hg at 13·8 l./min. During exercise it was