their 1940 population may have been poorer than that of our 1966 population.

The mere presence of bacteria within the oral cavity does not necessarily indicate that detectable humoral antibodies will be present. Even in cases of clinical cystitis due to *Escherichia coli,* only 6% of the patients showed appreciable antibody responses(10). But if pyelonephritis developed, then 82% of the patients showed humoral antibody responses(10). Hence we may anticipate that the humoral antibody responses to *T. microdentium* in man will be correlated with active infection. There is support for this view in the work of Steinberg, Socransky, Gerskoff and Weinstock(11). Using a different HA technic for detecting antibodies to *T. microdentium,* they found that none of five patients with normal gingivae had HA titers >10 while 8 of 9 with periodontal disease had titers of 10 to 40. Although no inhibition studies were reported, their results are consistent with the concept that antibody formation requires infection.

Summary. 1. Rabbits heavily immunized with *Treponema microdentium* develop high titers of both complement-fixing (CF) and hemagglutinating (HA) antibody. 2. Of 77 human sera, 5% had low titers of CF antibody and 12% had low titers of HA antibody to *T. microdentium.* 3. The patterns of antibody response in both the rabbits and patients indicate that the CF and HA antibodies are produced in response to different antigens of this spirochete.

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Quantification of Cellular Influx into the Croton Oil-Induced Pouch. Effects of Prednisolone.* (31791)

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A number of models have been developed to study inflammatory processes in both man and experimental animals. The modified Selye "granuloma" pouch of Roberts and Nezamis has been extensively used to assay for anti-inflammatory agents(1,2,3). Glenn and his associates analyzed the chemical composition of exudates obtained from pouches induced by the local administration of croton oil and d-α-tocopherol(3). The leukocyte content of these fluids has not, however, been examined. Moreover, the influence of anti-inflammatory agents on the cellular composition of the exudates remains unexplored. It was the purpose of these investigations to examine leukocyte influx into croton oil-induced pouches and to study the effect of locally administered prednisolone on the numbers and types of cells mobilized into this inflammatory site.

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Materials and methods. Male rats of the Long-Evans strain weighing approximately

200 g were used throughout these investigations. All animals were maintained on a diet of Purina Lab Chow and tap water *ad libitum*.

Three groups of animals were studied:
Group I. Croton oil-induced pouches were prepared on the backs of 12 rats using 0.5 ml of 1% croton oil as the irritant(1).
Group II. Pouches were prepared in a similar manner on 10 rats; each of these animals was injected daily with 5.0 mg prednisolone (Meticortelone, Schering) directly into the pouch.
Group III. For purposes of studying the effect of croton oil on another tissue compartment, 6 rats were injected intraperitoneally with 0.5 ml of 1% croton oil.

Peripheral leukocyte counts were made daily on free-flowing tail blood using the Spencer Bright-Line hemocytometer and 1% acetic acid as diluent. Three hundred cells were identified for peripheral differential determinations on slides treated with May-Grünwald stain. Hematocrits were determined daily using the standard microhematocrit method. Animals in Group III were autopsied five days after croton oil injection. Animals from Group I and Group II were sacrificed on day 6 after pouch formation, and the pouch exudate, peritoneal exudate, peripheral blood and bone marrow quantitated. The pouch fluid was aspirated and placed in a calibrated glass centrifuge tube containing a few crystals of dry heparin. Total leukocyte counts and hematocrits of exudates were determined. The collected pouch and peritoneal material was centrifuged at 500 rpm for 3 minutes, the supernatant poured off and the remaining button of cells diluted with 0.5 ml of homologous serum and smeared for differential analysis. The slides were treated with May-Grünwald stain and 500 cells enumerated. Peritoneal cellularity was determined by lavaging the peritoneal cavity of each rat with 10 ml of saline after the technique of Gordon *et al*(4). Slides were prepared as described above and 500 cells counted for differential analysis. Bone marrow cellularity and myelograms were determined for each rat using a modification of the technique of Fruhman and Gordon(5).

Results. Fluctuations in mean peripheral white blood cell counts (WBC); mean polymorphonuclear leukocytes (PMN), and mean mononuclear (mostly lymphocytes) elements (Mono) are given for the 3 groups studied in Fig. 1. A perceptible leukopenia was evidenced in all groups on day 1 following the administration of croton oil. The initial drop in peripheral WBC was due to a decline in circulating Monos, which is usually characteristic of a general stress phenomenon. On all subsequent days of the study, the numbers of Monos in the circulation were greater than starting values. Marked fluctuations in peripheral PMNs were apparent throughout these investigations. One day after croton oil injection, elevation in PMNs was noted in Groups I, II and III respectively. The maximum rise in mean circulating PMNs was seen 5 days after croton oil administration in Groups I, II and III. No appreciable alterations in peripheral hematocrit values in the 3 groups were observed over the time course of these investigations.

Prednisolone produced a marked reduction in mean pouch exudate volume and in the mean total cellularity of the pouch exudates (Table I). However, the mean cell content per milliliter of pouch exudate was not altered by the steroid. The leukocyte influx was differentially altered with prednisolone treatment which resulted in a relative neutropenia and mononuclear cell increase within the pouch. It is, therefore, apparent that the cellular composition of the exudates is not solely determined by the volume of tissue fluid accumulated in the inflammatory site. The data tend to support the view that both exudate volume and cellular composition are altered by prednisolone.

The mean peritoneal exudates of rats in Group I and Group II were comparable with respect to the numbers of cells lavaged, as well as cell types present (Table II). The mean cell number in the peritoneal cavity of rats receiving croton oil intraperitoneally (Group III) was found to be elevated over that seen in pouch induced groups. Appreciable numbers of PMNs were found in the peritoneal cavity of animals in Group III, whereas significantly few of these elements

were noted in Groups I and II (Table II).

Bone marrow cellularity and myelograms were normal in all groups studied.

Discussion. The presence in rats and other small mammals of local inflammatory sites has been reported by Glenn and his associates(6) to cause a marked increase in plasma inflammation units (proteins precipitable at 56°C). This elevation has been demonstrated by these investigators to be a reliable index of the existence of inflammation in the organism. They noted such rises in rats bearing pouches produced with either croton oil or d-α-tocopherol as the irritant. Our findings of a peripheral leukocytosis in rats injected with croton oil into the pouch or into the peritoneal cavity would tend to substantiate these observations that a peripheral manifestation (*e.g.,* elevated plasma inflammation units or peripheral leukocytosis) signals the presence of inflammation. The peripheral blood picture during the first eight hours after croton oil injection into the pouch was characterized by a marked leukocytosis with elevations in numbers of circulating PMNs and a decrease in circulating Monos. This indicates that croton oil is capable of inducing symptoms of stress and promotes physiological alterations at sites distant from its application.

Steroids and specifically prednisolone, when administered locally or systemically, are known to elicit marked increases in the circulating leukocytes(6-10). The percent change in peripheral leukocyte numbers of rats treated with prednisolone (Group II) was consistently greater than that seen in untreated animals (Group I). Peripheral leukocyte values, however, were most labile in Group III. The marked leukocytosis noted in these animals may have resulted from the appearance of tissue breakdown products or even the irritant in the circulation. The rate of egress of such leukocytosis-promoting material from the peritoneal cavity would be more rapid due to the high degree of vascularization. Thus, the leukocytosis, which is characterized by an increase in both PMNs and Monos, in Groups I and III, was in all likelihood caused directly or indirectly by the irritant; whereas in Group II it was influenced, at least in part, by the prednisolone.

Glenn and his coworkers were successful in reducing the volume of fluid in croton oil induced pouches by 99% after administration of 5 mg/day prednisolone directly into the pouch(3). Robert and Nezamis found that the volume of exudate in croton oil-induced pouches was proportional to the irritant(1). Injections of cortisol and cor-

TABLE I. Cellularity of Croton Oil-Induced Pouch Exudates Obtained 6 Days After Pouch Formation from Untreated Rats (Group I) and Rats Injected with 5.0 mg of Prednisolone Daily Directly into the Pouch. (Mean ± S.E.$_m$.)

Group	No. rats	Vol, ml	WBC/mm^3 × 10^{-3}	Total cells × 10^{-6}	Het	PMN × 10^{-6}	Mono × 10^{-6}	Eos × 10^{-6}	Mast × 10^{-6}
I	12	6.70 ± .39	5.89 ± .69	38.58 ± 4.90	6.70 ± .68	15.48 ±1.75	22.68 ± 3.89	1.20 ± .41	.01 ± .001
II	7	1.17 ± .39	6.46 ± .93	6.97 ± 1.84	14.57 ±3.02	.87 ± .19	6.04 ± 1.66	.06 ± .03	—

TABLE II. Cellularity of Peritoneal Exudates Obtained from Rats with Croton Oil-Induced Pouches (Group I); Rats with Croton Oil-Induced Pouches Treated with Prednisolone (Group II), and Rats Injected Intraperitoneally with 0.5 ml of 1% Croton Oil (Group III). (Mean ± S.E.$_m$.)

Group	No. rats	Vol, ml	WBC/mm^3 × 10^{-6}	Total cells × 10^{-6}	PMN × 10^{-6}	Mono × 10^{-6}	Eos × 10^{-6}	Mast × 10^{-6}
I	12	6.75 ± .20	3.45 ± .57	23.54 ± 4.27	.07 ± .04	16.48 ± 2.27	6.57 ± 1.96	.42 ± .08
II	7	7.98 ± .01	3.96 ± .08	31.55 ± 6.22	.02 ± .01	58.60 ± 3.20	12.20 ± 2.97	.51 ± .07
III	5	8.32 ± .10	6.80 ±1.50	56.70 ±13.19	8.40 ±2.50	44.13 ±11.10	4.15 ± 1.11	—

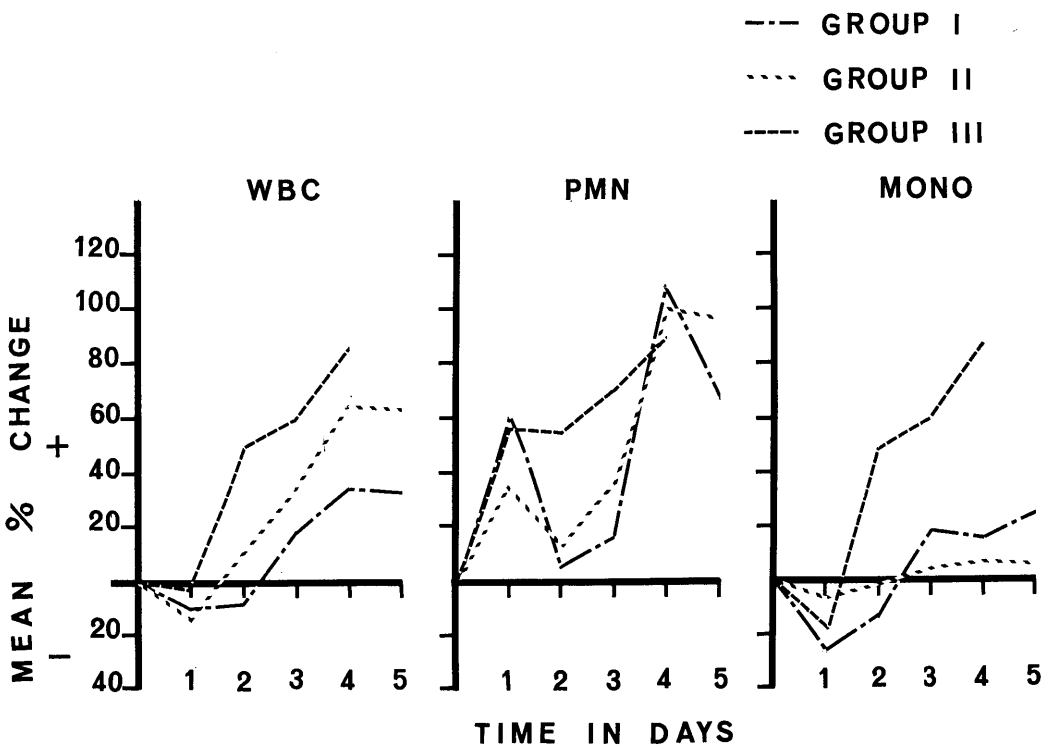

FIG. 1. Alterations in mean peripheral leukocyte counts of rats with croton oil-induced pouches (Group I); rats with croton oil-induced pouches treated with prednisolone (Group II), and rats injected intraperitoneally with 0.5 ml of 1% croton oil (Group III).

tisol acetate directly into the pouch reduced the volume of exudate formed. Pouch exudates were reduced in our experimental animals by 82.6% with the administration of 5 mg/day of prednisolone directly into the pouch for five days. The total exudative cellular content was comparably reduced by 82%; however, mean PMN values were simultaneously reduced by 94.4% despite increased numbers in the circulation. Prednisolone appears to influence markedly the cell influx as well as the volume of exudate formed in the croton oil-induced pouch. Despite the fact that there were proportionately greater numbers of Monos in the pouch exudate, the possibility that this agent acts to promote mobilization of mononuclear elements is unlikely.

From these data it appears that alterations in PMN influx into an inflammatory site constitute a sensitive index of inflammation. Furthermore, the effect on numbers of PMNs in an irritated site may well be used along with assessment of exudative volumes in the evaluation of the anti-inflammatory activity of test materials.

Summary. Quantitation of leukocyte influx into the croton oil-induced pouch in rats was performed, and the effects of 5.0 mg of prednisolone injected daily directly into the pouch determined. Prednisolone reduced the volume of the exudate as well as the total numbers of leukocytes mobilized into the irritated site. The decrease in the numbers of PMNs in the pouch exudate was greater than that seen for other cell types as well as for the reduction in exudate volume. The PMN content of the pouch exudate is suggested as an additional sensitive and reliable criterion for the assessment of anti-inflammatory activity of potential therapeutic agents.

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Effects of Sodium Alginate on Absorption and Deposition of Radioactive Cations in the Chick.*† (31792)

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A major problem involved in the use of cation-binding agents in removal of Sr is that Ca is removed concurrently and often to a greater degree. However, attempts have been made to use sodium alginate, a mucopolysaccharide from brown algae and Pacific kelp, as an agent for selective inhibition of the absorption of radiostrontium from the gut. Skoryna *et al*(1) found that sodium alginate reduced the uptake of Sr^{89} by 50-80% in the ligated intestine of rats without significantly affecting Ca^{45} absorption. Orogastric intubation and feeding the alginate were also reported to be effective in rats (2,3) and in humans(4). However, data are not available on dried kelp itself.

Materials and methods. To determine the effects of alginate on the preferential intestinal absorption of Sr^{89} and Ca^{45}, 20-day-old male chicks were distributed into 4 groups of 5 birds each, and fed a practical type ration supplemented with 0.5%, 1.0%, 2.0% sodium alginate, and 4.35% kelp (equivalent to 1.0% alginic acid), respectively. An additional group of 5 birds received a diet containing no alginate. All birds were given approximately 5 μc Ca^{45} orally 3 hours after they were placed on the experimental rations. Five other groups of 5 birds each were treated similarly, but were given approximately 5 μc Sr^{89} orally.

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The feces from each group were collected daily for isotopic analysis and at the end of the 14-day experimental period all birds were killed and the right and left tibiotarsus taken for analysis of the appropriate isotope. Both isotopic analyses were performed by the method of Creger *et al*(5) and all data were calculated by the computer method of Creger *et al*(6).

Results and discussion. Table I shows the percent of the total dose recovered in the tibiotarsus and the excreta from birds receiving single oral doses of Sr^{89} and Ca^{45}, respectively, and fed diets containing various amounts of sodium alginate or an equivalent amount of dried Pacific kelp.

The smallest amount of Sr^{89} was recovered in the tibiotarsus of birds which received 0.0% and 0.5% dietary sodium alginate. However, when alginate was included in the diet at the 1% or 2% levels, the deposition of Sr^{89} increased. Correspondingly the greatest excretion of Sr^{89} was by birds receiving no alginate and the excretion decreased as the alginate content of the diet was increased. Both Sr^{89} deposition in bone and Sr^{89} excretion in the groups receiving 1% and 2% alginate were essentially the same.

The greatest amount of the total dose of Sr^{89} recovered from tibiotarsus was from birds receiving 4.35% dried kelp. The amount actually recovered in the bones of the group receiving the kelp was more than