clature we refer to these substances as plasma Ac-globulin and serum Ac-globulin. The properties of the two are so similar that no difference has been detected, even though both have been obtained in concentrated form.

Thrombin is the substance which is responsible for the production of serum Ac-globulin, and calcium ion is not required for that purpose. The most active thrombin prepara-

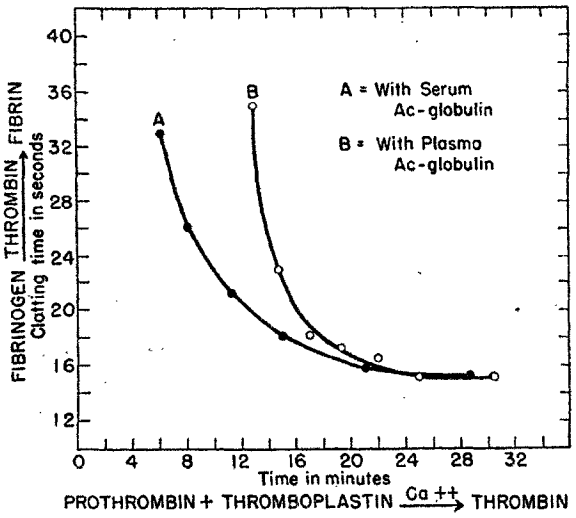


FIG. 1. Activation of purified prothrombin with serum Ac-globulin (curve A) and with plasma Ac-globulin (curve B) in the presence of excess thromboplastin and optimum calcium-ion concentration.

tions obtained to date have been added to large quantities of oxalated bovine plasma, and subsequently, concentrates of serum Ac-globulin have been obtained in quantity and quality equal to those obtained from bovine serum itself.

The function of Ac-globulin in the clotting mechanism can then be outlined by use of the following equations:

(1) Prothrombin + Thromboplastin $\xrightarrow{Ca^{++}}$ Thrombin

(2) Plasma Ac-globulin $\xrightarrow{\text{Thrombin}}$ Serum Ac-globulin

(3) Prothrombin + Thromboplastin $\xrightarrow[\text{Serum Ac-globulin}]{Ca^{++}}$ Thrombin

(4) Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin Clot

The clotting reaction is initiated by thromboplastin which comes from platelets and tissue juices. Some of the newly formed thrombin alters plasma Ac-globulin so that it becomes serum Ac-globulin. The latter intensifies the interaction of prothrombin and thromboplastin. Thrombin thus accelerates its own formation through an intermediate. This may be regarded as co-autocatalysis. These conclusions differ distinctly from those of Owren (*3*), but are in harmony with the old and well-known evidence presented in the literature to show that autocatalysis is involved in thrombin formation. This is, however, not autocatalysis but co-autocatalysis, because an intermediate is involved.

We have found that neither serum Ac-globulin nor plasma Ac-globulin can substitute for thromboplastin in the activation of prothrombin in the presence of optimum amounts of calcium ion.

The curves of Fig. 1 were obtained with the use of prothrombin prepared by $(NH_4)_2SO_4$ fractionation as described previously (*4*). This product possessed a maximum specific activity of 23,000 units/mg. of tyrosine. The activity was measured by the two-stage method (*7*). Plasma Ac-globulin was purified by the method briefly outlined (*5*). The same procedure was used for the preparation of serum Ac-globulin.

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# The Action of Pteroylglutamic Conjugates on Man[1]


SIDNEY FARBER, ELLIOTT C. CUTLER, JAMES W. HAWKINS, J. HARTWELL HARRISON, E. CONVERSE PEIRCE, 2ND, and GILBERT G. LENZ

*The Children's Hospital, Peter Bent Brigham Hospital, New England Deaconess Hospital, Boston,* and *Departments of Pathology* and *Surgery, Harvard Medical School*


In 1944, Leuchtenberger, Lewisohn, Laszlo, and Leuchtenberger (*4*) reported that a "folic acid concentrate" and a fermentation *L. casei* factor inhibited the growth of sarcoma 180 transplanted in female Rockland mice. Further studies by Lewisohn and his co-workers (*5*) in 1945 showed complete regression in about one-third of the single spontaneous breast cancers observed in three different strains of mice treated with daily intravenous injections of 5 μg. of fermentation *L. casei* factor. This substance was thought at that time to be folic acid; it is now known that it was a conjugate of folic acid, pteroyltriglutamic acid (*3*). Subsequent work showed that pteroylglutamic acid (folic acid), when tested under similar conditions, was not effective in producing regression of these breast cancers (*6*).

In 1944, Hutchings, *et al.* (*3*) reported the isolation of the fermentation *L. casei* factor. This compound was shown to be 60–80 per cent as active when assayed with *L. casei* and 2–6 per cent as active when assayed with *Str. faecalis* R as was the previously isolated liver *L. casei* factor, pteroylglutamic acid (*8*).

Degradative reactions have shown that the fermentation *L. casei* factor differs from pteroylglutamic acid in that the


[1]We are grateful for the cooperation of Y. SubbaRow and his colleagues in the research division of the Lederle Laboratories Division, American Cyanamid Company, who are responsible for the chemical research which forms the foundation of these studies, and to Benjamin Carey, who made available these substances for experimental trial. The compounds were furnished in the form of dry material, yellow-orange in color, in sterile vials, under the names *teropterin* and *diopterin*.

Thanks are due to the staffs of The Children's Hospital, the Peter Bent Brigham Hospital, and to Shields Warren and the staff of the New England Deaconess Hospital, Boston, for invaluable assistance and cooperation which will be acknowledged specifically in detailed reports to be published. The assistance of Elisabeth Blumenthal, R.N., is gratefully acknowledged.

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former compound contains two additional moles of glutamic acid (*1*). Consequently, pteroyl-γ-glutamyl-γ-glutamylglutamic acid (pteroyltriglutamic acid or *teropterin*) was synthesized and found to have microbiological activities identical with those of the naturally occurring fermentation *L. casei* factor (*2*). During the course of this synthetic work the compound pteroyl-α-glutamylglutamic acid (pteroyldiglutamic acid or *diopterin*) was also prepared and found to be only slightly active when assayed with *L. casei* and *Str. faecalis* R (*7*). This compound is not a naturally occurring substance. Its preparation indicated the possibility of further research with other members of the pteroylglutamic series.

The synthesis of these two compounds by SubbaRow and his co-workers made possible experimental clinical studies with new substances of the glutamic series of known chemical structure. Our decision to employ these compounds on patients with malignant disease was based on a critical analysis of the data in the cited reports of the animal experimental work by Lewisohn and his co-workers on the effect of the fermentation *L. casei* factor (now known to be pteroyltriglutamic acid).

It is the purpose of this note to report briefly some observations made in conjunction with the administration of these and closely related substances to 90 patients with malignant disease. Only those patients for whom established therapeutic procedures offered no hope of cure were selected for treatment with these compounds. This necessary restriction to patients with advanced neoplastic disease, most of them with metastases and many of them treated previously with X-radiation, makes difficult the interpretation of data and necessitates large numbers of observations. It is too soon to attempt any evaluation of the action of these substances on the course of neoplastic disease in man. This note will therefore be limited chiefly to a consideration of toxicity, dosage, method of administration, and certain general effects. Detailed clinical and pathological studies will be reported later.

This series includes patients with acute leukemia; astrocytoma; Ewing's tumor; carcinoma of the rectum, colon, stomach, cervix, prostate, pancreas, esophagus, bladder, breast, gall bladder, kidney, and ovary; Hodgkin's disease; lymphosarcoma; osteogenic sarcoma; ependymoma; spongioblastoma multiforme; seminoma; hypernephroma; leiomyosarcoma of the stomach; chondrosarcoma; epidermoid carcinoma of the pharynx and of the tongue; and embryoma of the kidney.

The patients varied considerably in age, 8 being under 3 years of age; 29 from 4 to 10; 4 from 11 to 20; 8 from 21 to 30; 10 from 31 to 50; 28 from 51 to 70; and 3 over 71.

The duration of treatment varied from a few days to 5 months; the average length of treatment was about 5 weeks.

After cautious initial trials were made, pteroyltriglutamic acid (*teropterin*) was administered in daily doses varying from 10 to 150 mg. intramuscularly and in other patients from 20 to 500 mg. intravenously. Pteroyldiglutamic acid (*diopterin*) was given in amounts from 50 to 250 mg. intramuscularly and from 20 to 300 mg./day orally. One patient received 19,000 mg. of pteroyltriglutamic acid over a period of 5 months, and 12,740 mg. were given intravenously to another in the space of 6 weeks, in both instances without evidence of toxicity.

On the basis of experience alone our present *initial* treatment calls for the administration of 20 mg. daily of either substance intramuscularly for one week, after which the dose is raised to 50 mg./day for two to three weeks longer. Decision concerning further experimental study is then made after the status has been evaluated.

Each substance was dissolved easily in from 1 to 8 cc. of saline for intramuscular or intravenous administration. When large amounts of the material were used intravenously, as large a volume as 20 cc. of normal saline was employed.

There have been no reactions following *intravenous* administration of either substance. No important local reactions following *intramuscular* injection of pteroyltriglutamic acid have been observed. Intramuscular injection of the diglutamic compound has been followed in some, but not in all, patients by moderate local reactions described as local burning and slight aching, lasting generally not more than several hours. This reaction was said to be usually no worse than that following intramuscular injection of penicillin. In no instance was there a local reaction which required special treatment.

Systemic reactions have not been observed. No important changes in pulse, respiration, or temperature were noted, nor have there been any significant long-term variations in blood pressure. There have been no allergic reactions of either major or minor degree. Detailed clinical laboratory studies were made; analysis of these data revealed no evidence of a deleterious action of the substances administered.

Twenty-seven of the patients with advanced neoplastic disease have died, and, of these, 13 were examined post mortem. Exclusive of 11 patients with acute leukemia from whom biopsies of bone marrow were obtained, there were 11 patients from whom biopsies of the tumor were obtained both before treatment was instituted and after treatment had been carried out for a period of at least a few weeks. Study of the gross and histological material available from these patients revealed no change in organs and tissues which could be regarded as a deleterious effect of the substances employed. In no instance was there any evidence of pancytopenia, agranulocytosis, degeneration of the kidneys, liver, or myocardium, or any suggestion of a polyarteritis.

The limited number and the short duration of these observations, and the possible role of psychotherapy, necessitate postponement of any conclusion concerning general effects upon the patient. In general, adult patients experienced improvement in energy, appetite, sense of well-being, and appeared to demonstrate less irritability and apprehension. In many instances, but not in all, such improvement might be ascribed to improved morale resulting from frequent visits, more medical attention to details of their complaints, and a definite impression that something more than usual was being done for them. In a few instances there was a definite diminution in pain which could be measured by a reduction in the amount of sedation or analgesia required.

Analysis of the collected data on the group as a whole showed that in a few instances conditions were such that a causal relationship was apparent between the administration of the glutamic compound employed and changes in the patient's condition or in the histological appearance of the tumor obtained at biopsy or at autopsy. In a larger group of patients with a clinical picture complicated by the use of more than one therapeutic agent (such as radiation therapy) in addition to the glutamic compound employed, changes were observed under conditions which suggested that it was the addition of the glutamic compound which played an

important part in their appearance. Examples of these changes were: temporary (several weeks) decrease in the size of multiple subcutaneous nodules of an amelanotic carcinoma; temporary (several weeks) decrease in size of metastases to the lung from a carcinoma of the testis; degeneration and necrosis which on two occasions was massive, as seen on pathological examination of tumors; reduction to normal on two occasions of several weeks each in the acid phosphatase level in the blood of a patient with multiple metastases to bone from a carcinoma of the prostate. Such changes have been by no means constant. They have occurred frequently enough, however, to warrant further experimental studies of the action of these and of related compounds on patients with cancer.

This preliminary report of the action of pteroyltriglutamic acid and pteroyldiglutamic acid on man reveals that these substances, as employed in these studies, are nontoxic and may be given either intravenously or intramuscularly. The absence of evidence of toxicity, as shown by clinical, laboratory, and post-mortem studies, and the ease of administration indicate that these substances are suitable for clinical use. No evidence has been presented in this report to suggest that these substances should be employed in the routine therapy of patients with cancer. Enough has been learned from these studies, however, to indicate that further investigation of the action of these and related compounds would be of interest.

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# IN THE LABORATORY

## A Mincing Apparatus for the Preparation of Embryo Extract for Tissue Culture[1]


Esther Carpenter

*Smith College, Northampton, Massachusetts*


In order to obtain more uniformity in the preparation of embryo extract, a number of substitutes have been suggested for the tedious method of cutting with scissors until pieces of tissue are too small to be identified. One of the simplest of these is the method suggested by Earle (*1*) in which a piece of monel metal screen of known mesh size is inserted in the base of a syringe, enough pressure then being exerted on the plunger to force the embryonic material through the mesh. This works satisfactorily with young embryos. If, however, one is using chick embryos of 10 or more days of incubation, two problems arise: (1) it is very difficult to exert enough hand pressure on the plunger to force the material through the screen; and (2) the increase in pressure is accompanied by danger of breakage of the syringe.

To circumvent these difficulties the equipment illustrated in Fig. 1 was devised and made of monel metal with the help of Russell Douglas, of the Physics Department. The tubular cup (A) is large enough to contain at least two 10-day chick embryos. At one end the cup is closed by a disc perforated by holes about 1 mm. in diameter. At the other end the inside of the cup is threaded to match the threads on the plunger (B). The latter is equipped with a horizontal handle by means of which the plunger can be screwed in far enough to force all the material in the tubular container through the holes in the base. For convenience, and also to avoid handling the equipment when sterile, a holder (C) was made which fitted around the cup and could be tightened by means of a screw in contact with a flattened area on the outside of the tubular container (A).

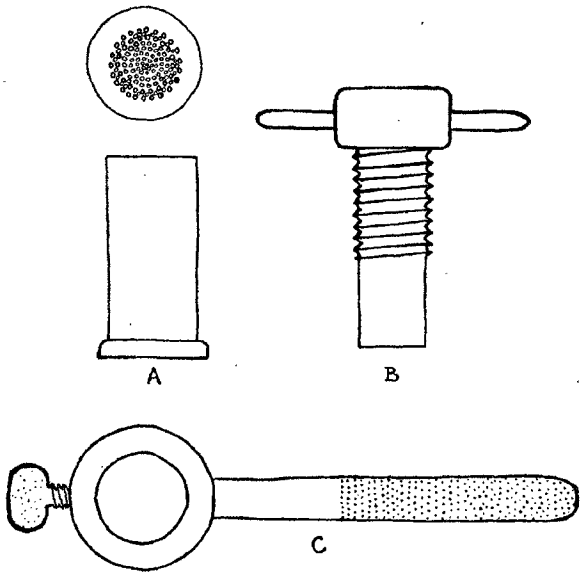


Fig. 1

This piece of equipment has proved very useful in our laboratory during the last few years. There is no danger of breakage, and the handle on the plunger and the screw arrangement make it possible to exert considerable pressure with a minimum of effort.

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[1] Contribution from the Department of Zoology, No. 216.