such as fish solubles, liver, and distillers' dried solubles were found to be inactive. However, it has been impossible to confirm the results of Novak *et al.* using mice. Likewise it is not known whether or not the postulated factor/s, is identical with that proposed by Ruegamer(9) or by Zucker and Zucker(10).

Summary. The addition of succinylsulfathiazole to low fat diets caused a growth retardation in mice although adequate quantities of all known accessory growth factors were fed. Normal growth resulted when fat, cottonseed meal, or rolled oats were added to the basal diet containing succinylsulfathiazole.

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Thromboplastic Potency Changes. A Result of Bacterial Action. (18321)

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Recent work in this laboratory has shown a significant factor in the potency deterioration of tissue extracts currently used as sources of thromboplastin to be associated with the activity of a bacterial contaminant, *B. subtilis*, in the thromboplastic suspensions.

It is the purpose of this paper to describe this effect and some properties of thromboplastic suspensions so altered.

Materials and methods. Rabbit brain and rabbit lung were used as sources of thromboplastic powder. Details of preparation of thromboplastic powders and their extraction to yield the final suspensions used, have been described elsewhere(1). Diluent for the thromboplastic suspension was 1:1 NaCl-$CaCl_2$ mixture (see below). Normal human oxalated plasma was employed (1 vol. of 0.1 M sodium oxalate to 9 vol. of freshly drawn whole blood). Ten per cent prothrombin plasma was obtained by diluting 100% prothrombin plasma with prothrombin-free plasma critically adsorbed(2) with $Ca_3(PO_4)_2$. All plasma was kept in an ice-water bath when not in use. Saline 0.85% NaCl and calcium 0.025 M $CaCl_2$ were used. Clotting time determinations: Our modification (1) of the Quick one-stage method was used, employing chemically clean glassware throughout. *B. subtilis* was cultured from a thromboplastic suspension which had undergone alteration upon incubation (see below). Routine bacteriological technic was employed. Difco brain-heart infusion with 2% agar was used as the plating medium. During the course of a day's routine clinical prothrombin determinations it was observed that the potency of the thromboplastic suspension decreased sharply during the period of the determinations. Observations made during a controlled study of the nature of the change in this batch of thromboplastic material was subsequently found to apply to all batches of brain and lung powders investigated.

Results. Brain thromboplastic suspension was incubated at 37.5°C for a period of 9 hours in a test tube open to air. During this time, oxalated plasma was kept in an ice-water bath and 0.1 cc portions removed as needed. Little change in clotting time occurred for the first 5 hours (Fig. 1). Between 5½ and 6 hours, however, there was a sudden, rapid prolongation of clotting time. The plasma, when tested at this time with a fresh thromboplastic suspension clotted in 12.4 seconds. The sudden prolongation in clotting time was therefore attributed to the loss in potency of the incubated brain thromboplastic suspension. A similar loss in potency was shown to occur with lung thromboplastic sus-

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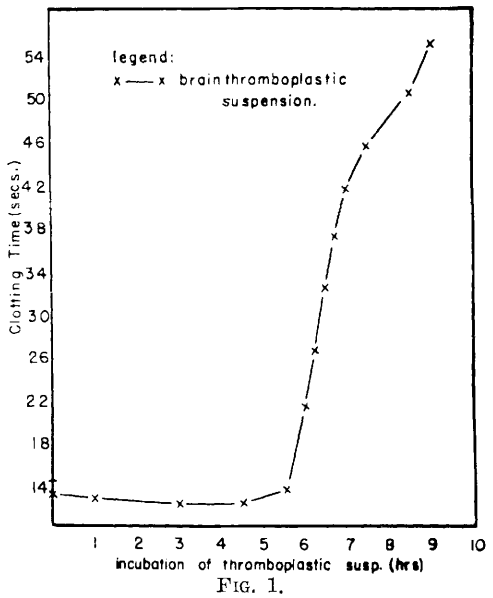

FIG. 1.

Characteristic curve showing suddenly prolonged clotting times obtained with normal human plasma when brain thromboplastic suspension is kept in open test tube at 37.5°C several hours.

pension in repeated experiments. The time of the "break" in the curve varied from 5 to 12 hours depending upon the sample of powder employed in making the thromboplastic extract. Such thromboplastic suspensions which have undergone apparent sudden loss of potency on incubation will hereafter be designated △ thromboplastic suspensions.

Prevention of deterioration. An attempt was made to shorten the prolonged interval before the sudden loss in potency by increasing the temperature of incubation 10°C. The unexpected finding that incubation at 47.5°C prevented the sudden loss of potency suggested that the observed effect might be the result of bacterial contamination. We therefore studied the effects of temperature and bactericides, simultaneously with bacteriological studies. It was found that the prevention of the sudden loss in potency of thromboplastic suspension could also be accomplished with phenol in final concentration of 0.25% as well as with "Mercurophen"* in final concentration of 0.02% (Fig. 2). Incubation at 47.5°C with subsequent incubation at 37.5°C caused a delay in the "break" in the curves. Concentrations of phenol and mercurophen lower than those given above also caused delay. Transferring thromboplastic suspension to 47.5°C after incubation at 37.5°C prevented or very greatly delayed the "break." However, once the "break" in the curve had started at 37.5°C, incubation at 47.5°C neither hindered nor stopped it.

During the course of incubation of brain thromboplastic suspension, clotting times with 100% prothrombin plasma were recorded. Simultaneously, 0.1 cc portions of brain thromboplastic suspension were removed and dilutions of 10^{-5}, 10^{-6} and 10^{-7} or 10^{-6}, 10^{-7} and 10^{-8} were made. Agar plate counts of bacterial colonies were made after 24 hours incubation at 37.5°C. The average count of 3 dilutions was recorded. The variation of the counts was somewhat large but the counts were all of the same order of magnitude (Fig. 3). There is a coincidence in the time of the sudden loss of potency of thrombo-

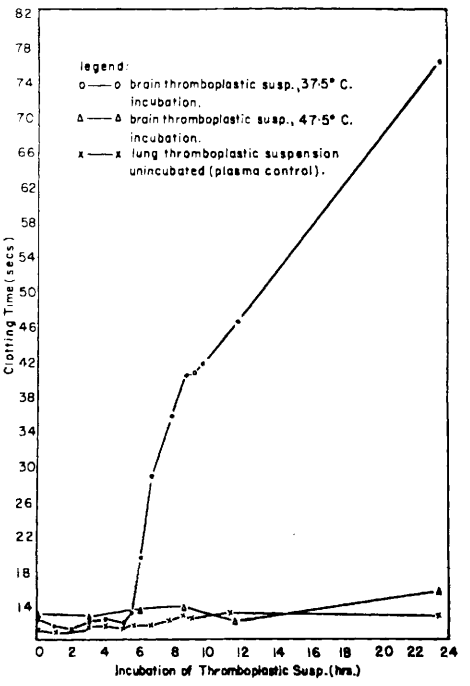

FIG. 2.

Showing that the sudden loss of potency can be prevented or greatly delayed by incubation of thromboplastic suspensions at 47.5°C. Phenol and Mercurophen are also effective.

* Sodium - oxy-mercury - ortho - nitro-phenolate. Sharp and Dohme.

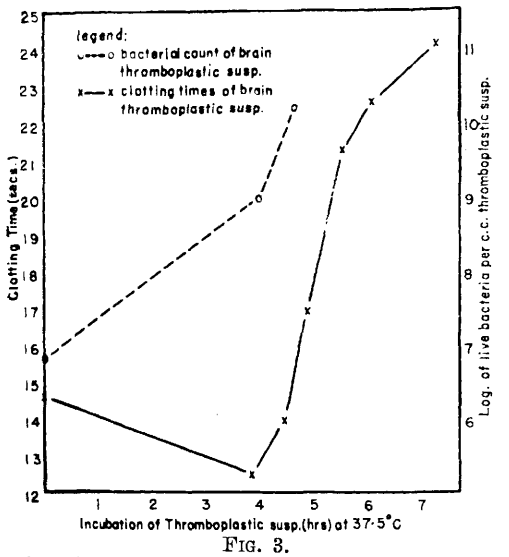

FIG. 3.

Showing the relationship between growth of organism (*B. subtilis*) in the thromboplastic suspensions and the sudden loss of potency.

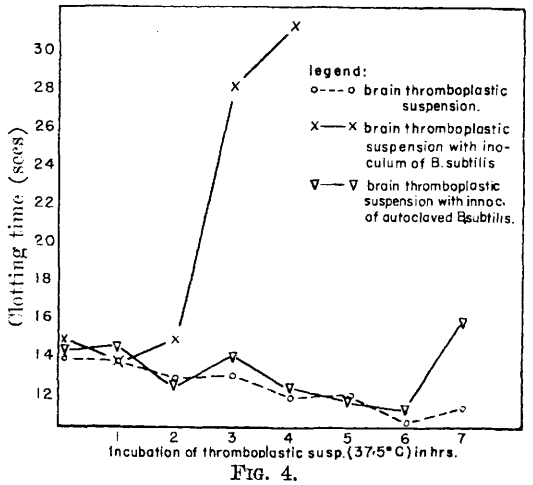

FIG. 4.

Showing that the sudden loss of potency of thromboplastic suspensions occurs sooner when *B. subtilis* is added initially to the suspension.

plastic suspension and the sudden increase in the colony count (number of organisms in the thromboplastic suspension). The organisms of the colonies which plated out of the △ suspension were uniformly found to resemble *B. subtilis*. This was confirmed by additional bacteriologic examinations.

To speed the occurrence of the "break" in the clotting time curve, it seemed advisable to add initially to the thromboplastic suspension, a concentrated suspension of an active form of the isolated *B. subtilis*. Accordingly, brain thromboplastic suspension was prepared and divided into 3 portions, one receiving no treatment, another receiving 0.3 cc of inoculum per 10 cc thromboplastic suspension, and a third receiving 0.3 cc of autoclaved inoculum per 10 cc of thromboplastic suspension. These 3 samples were incubated at 37.5°C and clotting times of each with 100% prothrombin plasma were determined. The data are given in Fig. 4. The inoculum of living organisms drastically reduced the time for the "break" in the curve. The clotting times obtained with the sample receiving autoclaved inoculum were indistinguishable (in the interval investigated) from those obtained with the untreated portion. The reduction of time for the break in the curve is even more striking if one incubates the inoculated thromboplastic suspension in vessels which allow greater air-liquid interfaces (*e.g.* Erlenmeyer flasks). Thus the sudden loss of potency of these thromboplastic suspensions appeared to

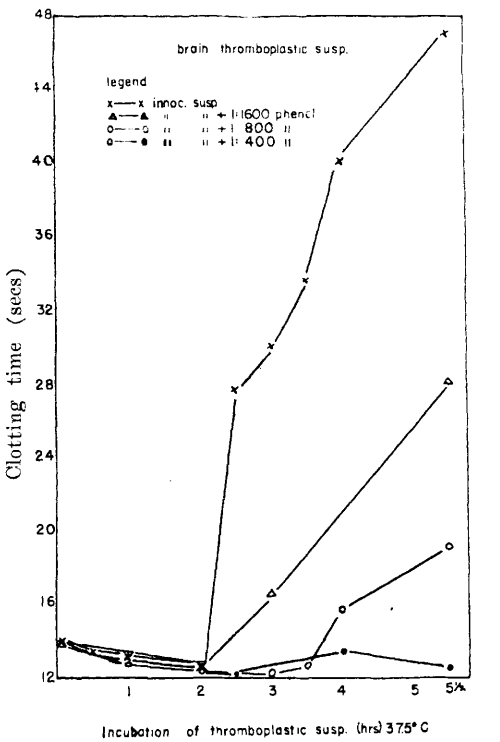

FIG. 5.

Showing the effect of various concentrations of phenol upon sudden loss of potency of preinoculated (*B. subtilis*) thromboplastic suspensions.

be associated with the vital activity of the organism.

Having thus reduced the interval before the sudden loss of potency it seemed of interest to determine the effects of phenol on such an inoculated suspension. Fig. 5 summarizes the data. Again it is seen that a final concentration of 1:400 (0.25%) phenol prevented the sudden loss of potency in the interval investigated. Lower concentrations of phenol were decreasingly effective.

Some aspects of the functioning of the △ *thromboplastic suspensions.* (1) Using unaltered (pre △) thromboplastic suspension, the clotting times of 100% and 10% prothrombin plasmas were determined at 5 minute intervals in the neighborhood of the "break" in the curve. The "break" in the curve was found to be simultaneous for both plasmas. (2) The clotting times of dilutions of △ suspensions were determined with 100% and 10% prothrombin plasmas. The clotting times for both 100% and 10% prothrombin plasma increased initially and progressively upon diluting the △ thromboplastins. (3) Prior to incubation, a portion of thromboplastic suspension was removed and refrigerated. After the incubated portion had undergone the sudden loss in potency (△ suspension) its clotting time with 100% prothrombin plasma was recorded. The refrigerated, unincubated portion of thromboplastin-$CaCl_2$ was then diluted until its clotting time with 100% prothrombin plasma was the same as that of the △ suspension. (The diluted, unincubated portion of thromboplastin is hereafter referred to as the dilution analogue.) In table I are shown the clotting times of △ suspensions of brain and lung and their dilution analogues on 100% and 10% prothrombin plasma. Though the 100% prothrombin clotting time for △ and dilution analogue suspensions were made to coincide, the 10% prothrombin clotting times of the △ suspensions are markedly longer than those obtained with their dilution analogues.

The results of this study have been found to hold true for lung thromboplastic suspensions as well.

Discussion. These data clearly demonstrate that the activity of *B. subtilis* caused a sudden loss in potency of brain and lung thromboplastic extracts. Preliminary experiments which indicate a separable enzyme-like material as the causative agent will be reported shortly. It is not yet possible to say whether the effect (apparent loss of potency) was due to elaboration of inhibitor, destruction of activator, or both. The data showing concomitant rise in clotting time with 100% and 10% prothrombin plasma can be interpreted as evidence against the destruction of activator. The reasoning is as follows: If activator were destroyed, the sudden rise in clotting time with 100% prothrombin should precede the sudden rise in clotting time with 10% prothrombin. This is true because the destruction of activator should result in a sub-optimal concentration of activator for 100% prothrombin plasma earlier than for 10% prothrombin plasma. Similarly, the clotting time data obtained with dilutions of thromboplastic suspension argue against the elaboration of a type of inhibitor previously described(2) since clotting times of 100% as well as 10% prothrombin increased steadily when tested with more dilute thromboplastic suspension. Had such an inhibitor been elaborated, one would have expected an initial decrease of 10% prothrombin clotting time upon thromboplastic dilution(2).

TABLE I. Longer 10% Prothrombin Clotting Time with △ Thromboplastin than with Diluted Uninoculated Thromboplastin of Same Potency on 100% Prothrombin.

	BTP		LTP	
	△ suspension	Dilution analogue	△ suspension	Dilution analogue
100% Pt.	31.0	30.3	18.7	17.8
10% Pt.	77.0	35.3	74.0	35.0

Conclusions. A resumé of results thus far indicates that a sudden loss of potency of thromboplastic suspension occurred upon open-air incubation at 37.5°C which could be prevented or significantly delayed (up to 24 hours at least) with 47.5°C incubation or with phenol or "Mercurophen." This sudden loss of potency was coincident with the log. growth phase of an organism isolated from

the thromboplastic suspension and identified as *B. subtilis*. Inoculation of thromboplastic suspension with a physiologically active form of *B. subtilis* drastically reduced the interval before the abrupt prolongation of clotting time. This also could be prevented (up to 7 hours at 37.5°C at least) with 0.25% phenol. The fact that *B. subtilis* can alter the apparent thromboplastic potency of tissue suspensions seems to have practical value as well as theoretical promise. From the practical aspect, the utilization of thromboplastic suspension can be extended by simple addition of phenol in cases where the potency loss is due to bacterial action. From the theoretical aspect, it would appear that the △ suspensions have an altered activator-inhibitor content. Such suspensions are amenable to investigation leading to possible concentration, or separation of activator or inhibitor. It is apparent from these preliminary observations that the nature of the changes of the incubated thromboplastic suspensions are yet to be explained. It is clear, however, that the marked changes induced by this bacterium and controlled by simple conditions offer new methods of investigating the content and nature of thromboplastic materials without resorting to drastic physicochemical treatment.

Summary. Data have been presented which show that a bacterial contaminant identified as *B. subtilis* caused a sudden deterioration in potency of brain and lung thromboplastic suspensions, which could be prevented by various agents. Some aspects of the functioning of the altered thromboplastic suspensions are presented and their significance discussed.

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Effects of Thyroidectomy and of Thiouracil on Adrenal Weight and Ascorbic Acid. (18322)

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It has been demonstrated that thyroid substances induce an enlargement of the adrenal glands(1,2), whereas thyroidectomy, or the administration of goitrogenic substances, results in a decrease in adrenal size(2-4). These alterations in adrenal size are restricted to the cortex of the gland. Long(5) and Sayers and Sayers(6) have reviewed the recent wide use of adrenal ascorbic acid content as an index of adrenocortical activity. The changes in adrenal ascorbic acid following adrenal stimulation or depression are also confined to the cortex. The effects of thyroid material on adrenal size and ascorbic acid content have already been reported by Sure and Theis(7) and by Wallach and Reineke(8). The present investigation was undertaken to determine the effects of hypothyroidism on the adrenal weight and adrenal ascorbic acid concentration of the male rat. Since it has been shown by Gordon, Goldsmith, and Charipper(9) that thyroidectomy and thiouracil exert somewhat

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