Conclusion

Childbirth is being conducted in this manner in many parts of the world. There is no evidence yet that any school which has applied the psychosomatic approach to labour has withdrawn. No man who has understood the emotional has returned to the purely physical conduct of childbirth. There is more in this than the production of the child. At present there is a great wave of fear in the United States of America that the psychological state of married women is so altering that a factor is creeping into society which in a few generations may have a deleterious effect upon the whole nation. Two years ago—when I was in America—I found that the “mind of woman” received little or no care either before or during labour. Now, however, the importance to the mother and baby of this approach to childbirth has been accepted and demonstrated by Professor Herbert Thoms, of Yale University; Dr. Blackwell Sawyer, of New Jersey; Drs. Volmer and Marsh, of San Francisco; the Maternity Center Association, of New York; and others who have adopted these procedures. Their recent publications bear out with remarkable accuracy the conclusions drawn from the work in this country. Complete anaesthesia or unconsciousness in normal childbirth is an offence against Nature. Children who have been born according to the laws of Nature will be evidence of its psychological value as they grow to maturity. It will be easy to recognize those who were born with instinctive maternal guidance in the first few moments of their lives. These factors cannot be overlooked.

In a series of cases recently reported by the first great American university to adopt the procedure here outlined it was stated that 80% of all women—including abnormal labours—were fully conscious of all the sensations of the birth of their babies. I have found that in over 90% of uncomplicated births the women refuse analgesic or anaesthetic. The normal healthy woman of to-day, with a normal healthy labour, will tell you that the birth of her child was “a joy she would not have missed for anything.” She sympathizes with those whom circumstances forced into insensitiveness or oblivion at that moment.

NON-SPECIFIC EFFECTS OF IMPURE PENICILLIN

BY

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It has occasionally been reported during the last three years that impure penicillin is a more effective chemotherapeutic agent than the purified crystalline material. This view was based on the laboratory study of various penicillin samples, using either the protection of animals (Hobby *et al.*, 1946, 1947; Welch *et al.*, 1947; Miller *et al.*, 1948) infected with streptococci, *Bact. typhosum*, or salmonella or the action on the motility of *Treponema pallidum* (Dunham and Rake, 1945). These observations seemed to indicate the presence in crude preparations of penicillin of a factor that increases its antibacterial action; this factor appeared to have so marked an effect on the action of penicillin that the term “enhancement factor” was thought justifiable. This reason probably also explains the suggestion, made in a recent annotation (*B.M.J.*, 1948), to take the retrograde step of reverting to the earlier production methods and the use of impure penicillin.

It should be noted, however, that the data available explain neither the nature of the “enhancement factor”

nor its action (Welch, 1947; Hobby *et al.*, 1947; Fischbach *et al.*, 1947; Pratt *et al.*, 1948). The individual observations are scanty and occasionally conflicting, but this much is certain, that the “enhancement factor” in the impurities is heat-stable, dialysable, and active in small amounts if added to purified penicillin G, K, or dihydro F (Welch, 1947; Hobby *et al.*, 1947). The issue is of importance to the whole problem of presentation, for penicillin may be distributed either as a pure product with specific pharmacodynamic properties or as a crude one in which largely unknown impurities are left. An investigation was therefore undertaken to determine (1) what part the impurities play in the activity of crude penicillin, and (2) the mechanism of any such activity.

With these objects in mind the main work of Welch *et al.* (1947) was first repeated, but using, on a units-per-weight basis, smaller doses of penicillin—just sufficient to protect mice against lethal infections with *Bact. typhosum* or streptococcus, which are those commonly employed in protective trials.

Materials and Animals Used

In this paper we are concerned with a substance or substances that may be present in certain samples of penicillin and are different from the essential therapeutically active constituents. It is therefore important to describe as precisely as possible the penicillin samples used in the experiments. There is some confusion in the penicillin literature owing to the various descriptions of materials used in laboratory and clinical investigations, descriptions that include such terms as “crystalline,” “pure,” “purified,” “crude,” “yellow,” “white.” It has been thought best to follow as closely as circumstances permit the descriptions accepted in this country for the purposes of the Therapeutic Substances Act.

Two preparations were used, differing in “purity.” The first was “penicillin sodium salt,” containing not less than 80% of penicillin G (penicillin II, benzyl penicillin); the other 20% presumably consisted partly of penicillin and partly of material devoid of penicillin activity. It varied in colour from yellow to almost white, according as the impurities contained more or less of pigmented substances derived from the mould. The other material used was “crystalline penicillin G sodium salt”: it contained at least 95% of penicillin G and was a white macro-crystalline product.

For brevity the first type of product is referred to as IP and the second as CPG. The activities in international units were in conformity with accepted standards: the IP batches all had about 1,200 units per mg., the CPG 1,600 or over.

The mice used in the experiments were albinos of both sexes from an inbred colony derived from ancestors of the Strong A2 line. They weighed from 18 to 20 g. at the time of test. Rabbits, of no special strain, were used at body weights of 2.5 to 3 kg.; they received intramuscular injections of 10,000 units of penicillin.

Methods of Infection.—The procedure, including the method of infection, was similar to that of Welch *et al.* (1947), but differed from that of Hobby *et al.* (1947). Two different pathogens were used. A virulent strain of *Bact. typhosum*, having for our mice an LD50 of about 40,000,000 organisms, was injected, at ten times the LD50 of a 24-hour culture, by the intraperitoneal route. The other strain used was *β-Streptococcus haemolyticus* at ten times the LD50 of a 24-hour culture (blood agar), also intraperitoneally. Penicillin injections were given intraperitoneally in the morning, followed two hours later by the bacterial suspension. Treatment was given twice daily for four days and the number of mice surviving after seven days was recorded.

Inactivation.—For control purposes all IP or CPG solutions were inactivated with a penicillinase preparation from *Bacillus subtilis* (Ungar, 1944) and then heated at 80° C. for 30 minutes.

Comparison of IP and CPG

Table I gives the results of some comparisons of the two types of penicillin preparation with both kinds of infection. IP (K283) was a fairly old batch of material. The CPG was material from current production. In the experiment recorded in Table I, moreover, the batches CPG 5015 and CPG 5207 consisted of high-potency material made from a part of the same metabolism fluids that yielded, respectively, batches IP 5015 and IP 5207 of less-purified product. Thus the latter may be regarded as consisting of the former together with the natural impurities. A number of experiments like the one summarized here (but not involving the use of similarly comparable batches of IP and CPG) were carried out during a period of some 12 months with 18 other production batches of penicillin IP.

TABLE I

Substance	Penicillin Dose (i.u.)	Infection	No. of Animals Used	No. of Survivors
CPG	10	Streptococcus	36	24
CPG + IP K283 inactivated	10	"	36	27
CPG	100	Bact. typhosum	36	5
CPG + IP K283 inactivated	100	"	36	15
CPG	50	"	12	0
CPG + inactivated IP 5015	50	"	12	2
IP 5015 + inactivated CPG 5015	50	"	12	2
IP 5207 + CPG 5207	50	"	12	2
IP 5015	100	"	24	12
	450	"	24	23
CPG 5015	100	"	24	8
	450	"	24	19
Control	—	"	12	0

From these experiments it would appear that mice treated with IP are sometimes better protected than mice treated with the same dose of CPG. The difference between their action in mice infected with streptococcus was not significant; indeed, in some tests mice treated with CPG showed a slightly higher survival rate than mice treated with IP. But our experiments confirmed the superior effect of IP in preventing *Bact. typhosum* infection of mice.

Blood Levels in Rabbits

The next series of experiments was designed to find out whether the estimation of blood levels of penicillin in rabbits after injection of IP would give an indication of the presence of the enhancement factor, as had been suggested by Welch (1947). Blood levels were estimated at 15-minute intervals for the first hour and then half-hourly. The rate of urinary excretion of penicillin was also estimated, to determine whether the less-pure penicillin had the effect of decreasing urinary excretion, as this might partly explain any higher penicillin blood levels.

All the penicillin samples which showed an enhanced activity in typhoid-infected mice were tested, as well as ordinary routine batches.

For each batch a group of four rabbits was used. The tests showed that the type of penicillin tested had no bearing on the levels of penicillin in the blood. Maximum levels were reached after 30–45 minutes; the levels began to fall after 60 minutes, reaching zero after three to four hours, irrespective of the kind of penicillin injected. It was found, however, that the levels of penicillin in the blood differed in individual rabbits, sometimes very considerably. This was apparent in two experiments in which groups of four rabbits were alternately injected with the same two batches of CPG and IP. On the first occasion the batch of IP gave higher blood levels in three of the four rabbits after 60 minutes than did the CPG. In the

second test the first group of rabbits were injected with CPG and gave higher blood levels than the other group now injected with IP. The blood levels clearly depended to a large extent on the individual rabbits. This observation is already well known not only to laboratory workers but also to clinicians, who often record a wide range of penicillin blood levels in patients receiving the same dose of penicillin.

The amount of penicillin excreted in the urine of the rabbits was estimated during the day on catheter specimens and at night on samples collected in metabolism cages. The rate of excretion of penicillin in the urine and the amount of penicillin excreted over 24 hours did not show any difference with the two types of penicillin; with either, between 40 and 90% was accounted for in the urine after 24 hours.

Effect on General Resistance

As the increased effect of IP in our material could be demonstrated with *Bact. typhosum* infection but not with streptococcus, it seemed possible that if the blood levels of penicillin in mice behave similarly to those in rabbits we were here concerned less with the bacteriostatic action of penicillin, which should be "enhanced" similarly against both types of infection, than with an effect on the host, whose neutralization of bacterial endotoxin is being stimulated by the IP. This notion is supported by the fact that the enhancement effect can be shown if the mice are pre-treated with IP; the optimal response occurs if the IP or the impurities present in IP plus CPG are injected into the peritoneal cavity before an injection of the infecting organism. In this event the impurities would be producing just the opposite effect to that of an intraperitoneal injection of mucin-coated bacteria, which enables micro-organisms of low pathogenicity (mainly endotoxin-containing bacteria) to cause a fatal infection in otherwise resistant animals (Miller, 1933). It is appropriate to mention that this effect of mucin is due to several factors, such as the inhibition of phagocytosis and an increased permeability of the barrier between the abdominal cavity and the blood stream (Olitzki, 1948).

To demonstrate, therefore, that the effect of the impurities is on the host rather than directly on the bacteria, we made a few experiments to compare any stimulating effect of the IP with that of colloidal substances known to have such an effect. For this purpose calcium oleate (a suspension of 0.75% calcium oleate in 0.5% gelatin) was used, and the experiments were performed on similar lines to those with IP and mice infected with *Bact. typhosum*. The mice were injected intraperitoneally in the morning with 0.05 ml. of calcium oleate and infected two hours later. The results are shown in Table II.

TABLE II

Substance	Dose in Units	No. of Mice Surviving out of 12
CPG + inactivated IP	100	9
CPG + CPG	100	5
CPG + calcium oleate	100	12
IP	100	8
CPG	100	4
Calcium oleate	—	2
Control mice	—	0

Although the number of mice in each group was not large the results point to a positive enhancement effect of IP, whether inactivated or not, as well as of calcium oleate. The differences between the number of animals protected by CPG alone (4) and by CPG with inactivated IP (9) or with calcium oleate (12) are statistically significant at the 5% level.

Effect of Different Injection Routes

The method of treatment was next altered. One group of mice was treated intraperitoneally with CPG along with inactivated IP or calcium oleate. A second group was treated with CPG intraperitoneally and inactivated IP or calcium oleate subcutaneously. A third group was treated with inactivated IP or calcium oleate intraperitoneally and CPG subcutaneously (see Table III).

TABLE III.—50 Units of Penicillin Into Each of 12 Animals

Substance	Route of Administration	No. of Mice Surviving			
		A	B	C	Total
CPG + inactivated IP ..	Intraperitoneal	7	6	6	19
CPG + calcium oleate ..	"	6	6	5	17
CPG ..	"	11	10	6	27
Calcium oleate ..	Subcutaneous				
CPG ..	Intraperitoneal	10	10	6	26
Inactivated IP ..	Subcutaneous				
CPG ..	"	4	3	4	11
Calcium oleate ..	Intraperitoneal				
CPG ..	Subcutaneous	2	3	3	8
Inactivated IP ..	Intraperitoneal				
CPG ..	"	4	—	—	4
IP ..	"	8	—	—	8
Control ..	—	0	—	—	0

A, B, and C indicate contemporaneous tests.

Intraperitoneal injection of double doses (100 i.u.) of IP or CPG, each into 12 mice, produced about 50% protection; 12 unprotected negative controls all died.

From these experiments it seems justifiable to conclude that the simultaneous intraperitoneal injection of CPG and IP, or of CPG and the colloidal suspension, raises the survival rate of the infected mice above that of mice treated with CPG alone at the same dose level. It seems also that the subcutaneous injection of IP (or of the colloidal suspension) increases the effect of intraperitoneally injected penicillin above that produced by the IP and the penicillin injected at the same time by the intraperitoneal route.

Other Colloidal Agents

We also performed an experiment in which other colloidal suspensions, such as colloidal gold or copper (Crookes's "collosol"), were used in place of colloidal calcium oleate or IP. Sixteen hours before infection 60 mice were treated with 0.05 ml. of the colloidal suspension subcutaneously and 200 units of CPG intraperitoneally. Infection with *Bact. typhosum* by the intraperitoneal route followed, and treatment with the colloidal suspension subcutaneously and the penicillin intraperitoneally was given twice daily for four days. In this experiment, therefore, the period of pre-treatment was prolonged to 16 hours, and the treatment itself began about two hours after the infection.

The results after seven days showed that about 30% of the mice survived if pre-treated with the colloidal suspension or IP, but none survived if the colloidal suspension was given two hours after the infection. An injection of IP had an effect similar to that of pre-treatment with the colloidal suspensions, whereas CPG at the same dose as the IP protected only about 10% of the mice.

Leucocytosis in Peritoneal Fluid

The enhancing action of IP on CPG in the previous experiments can be partly explained by the results of the following investigation.

In the preliminary experiment with mice it was found that the normal amount of free peritoneal fluid was very small and that it varied considerably from mouse to mouse. It was therefore impossible to make even an approximate estimation of the leucocyte count in the peritoneal fluid. We therefore injected into the peritoneal cavities of mice

2 ml. of normal saline, killed the mice 40 minutes later, and estimated the number of leucocytes in the peritoneal fluid by the routine method of white-cell estimation in the blood, using 1% acetic acid as the diluent. The average number of leucocytes found was about 900–1,200 per c.mm. of the diluted peritoneal fluid, or a total leucocyte count in the peritoneal fluid of about 1.8–2.5 millions, assuming that the leucocytes were evenly distributed.

Having established the normal leucocyte count, we investigated the effect of intraperitoneal injections of calcium oleate, IP, and CPG on it. Groups of three mice were injected intraperitoneally with 2 ml. of saline containing one of the three substances. One hour later the mice were killed, the peritoneal fluid was removed, and the leucocyte count was estimated, with the results shown in Table IV.

TABLE IV

Substance	Amount	Mean Value of Leucocytes (3 mice)
IP ..	200 units	2,300/c.mm.
IP inactivated ..	200 "	1,860/c.mm.
CPG ..	200 "	1,200/c.mm.
CPG inactivated ..	200 "	1,260/c.mm.
Calcium oleate ..	0.05 ml.	1,430/c.mm.
Saline control ..	2 ml.	960/c.mm.

In a second experiment, in which groups of six mice were used, the leucocyte counts in the peritoneal cavities were estimated over a period of three hours after the intraperitoneal injection of IP and CPG, with the results shown in Table V.

TABLE V

Substance	Amount	Mean Value of Leucocytes (6 mice)	
		After 1 Hour	After 3 Hours
IP ..	200 units	2,000/c.mm.	9,300/c.mm.
CPG ..	200 "	1,075/c.mm.	5,400/c.mm.
Saline control ..	2 ml.	1,066/c.mm.	2,800/c.mm.

From these results the justifiable conclusion seems to be that intraperitoneal injection of IP causes a pronounced local leucocytosis in the peritoneal cavity. This leucocytosis probably results in an increased phagocytosis of the bacteria subsequently injected into the same cavity. This stimulated phagocytosis may be one of the factors contributing to the "enhancement effect" of IP.

Discussion

These experiments indicate that IP has a greater protective effect than CPG on mice infected with *Bact. typhosum*. This increase in activity was about 20–40%—i.e., 70 units of IP have the same effect as 100 units of CPG. These results confirm the reports of Welch *et al.* (1947) and of Hobby *et al.* (1947). In the results of treatment of streptococcal infections, no appreciable difference between our two types of penicillin was recorded. The more pronounced effect of IP in mice infected with *Bact. typhosum* is also consistent with the finding of Miller *et al.* (1948) that IP has a greater neutralizing effect on bacterial endotoxin (from *Salmonella aertrycke*) when injected intraperitoneally. The increased leucocytosis in the peritoneal cavity after injection of IP is probably a contributory factor in suppressing the endotoxin-containing bacteria.

We have also found that the protective action of CPG in infected mice could be increased by simultaneous administration of colloidal suspensions of calcium, copper, or gold. The method of action of the colloidal suspensions in the presence of CPG may be similar to that of the impurities in penicillin—through increased leucocytosis in the peritoneal cavity. This finding indicates that the "enhancement" effect of the impurities in IP is possibly due to activation of certain defence mechanisms in the

host rather than to any direct action of the impurities on penicillin itself. This would explain why the enhancement effect cannot be demonstrated *in vitro*, and rules out any direct synergistic effect of the impurities on penicillin. We were not able to confirm that the enhancement effect of the impurities caused the higher penicillin blood levels reported by Welch (1947).

There are as yet no reliable clinical data to demonstrate that less-pure penicillin (from modern deep culture production) has any therapeutic advantages over pure products. Early favourable reports are based on observations with batches of uncertain penicillin content used in the treatment of venereal diseases, mainly gonorrhoea, which are known to respond even to small doses of penicillin. From the pharmacological point of view all the arguments are in favour of using the purest penicillin. It is better tolerated in high doses by sensitive tissues like those of the central nervous system and eye than are cruder products; it has less often been reported to produce allergic sensitization; and it is more stable.

Summary

It has been found that highly purified samples of penicillin G sodium salt were not so effective as less-purified material in protecting mice against infection with *Bact. typhosum*, but that the difference was not demonstrable with streptococcal infections. A suggestion is advanced that the enhancing effect of "crude" penicillin may be partly due to increased leucocytosis in the peritoneal cavity after injection of the impurities present, and it has been shown that certain colloids do produce this leucocytosis and the "enhancement" of purified penicillin.

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NEEDLE BIOPSY OF THE LIVER WITH SPECIAL REFERENCE TO A MODIFIED GILLMAN TECHNIQUE

BY

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In 1833, at St. Bartholomew's Hospital, Stanley punctured the liver in a case of suppurative hydatid disease. Ehrlich (Frerichs, 1884) obtained liver tissue by aspiration through a needle in research on diabetes, and Lucatello (1895) used a similar procedure for diagnosis. These early reports were followed by studies employing variations of the aspiration technique, but they were largely unsatisfactory. The present status of liver biopsy dates from the work of Iversen and Roholm (1939), and since then its value has become widely recognized.

Indications and Contraindications

The following circumstances have been judged to indicate the use of liver biopsy:

1. Jaundice.
2. Hepatic enlargement of unknown cause.

3. Malignant disease when hepatic metastases are suspected.
4. Hepatic disease in which precise diagnosis is otherwise difficult: fatty infiltration, acute, subacute, and chronic hepatitis.
5. Obscure disorders possibly due to systemic diseases accompanied by morbid changes in the liver, when other methods of diagnosis have proved inconclusive: sarcoidosis, amyloidosis, haemochromatosis, Gaucher's disease, abdominal reticuloses, aleukaemic lymphatic leukaemia, glandular fever, kala-azar, and schistosomiasis.
6. Portal hypertension—to determine whether the obstruction is intrahepatic and whether lipotropic therapy is required before operation.
7. Constrictive pericarditis—to determine the extent of hepatic damage before operation.
8. The treatment of patients with fatty infiltration, smouldering infective hepatitis, or insulin-resistant diabetes.

The contraindications may be listed as follows:

1. Haemorrhagic states, failing to respond to therapy.
2. Marked anaemia.
3. Absence of superficial liver dullness.
4. Senility.
5. Lack of co-operation of the patient.
6. Refusal of the patient to enter hospital. Watson (1948) reports that some persons are carrying out liver biopsy as an ambulatory procedure.
7. The suspicion of liver abscess, hydatid disease, or active cholangitis.
8. Absence of specific indication.

Mortality

There is a widespread opinion that liver biopsy is too hazardous to be adopted as a standard investigation, but this seems to be based upon mortality figures which include series before the discovery of vitamin K. The following are the death rates recorded in modern series:

	Deaths	Biopsies
Gillman (1948)	1	1,000
Sherlock (McMichael, 1948)	2	400
Roholm, Krarup, and Iversen (1942)	2	297
Volwiler and Jones (1947)	1	278
Beek and Haex (1943)	0	200
Davis, Scott, and Lund (1946)	0	79
Herrera and Pardo (1947)	0	72
Hoffbauer (1945)	0	65
Baron (1939)	1	48
Beierwaltes and Mallory (1946)	0	30
Total	7 (0.28%)	in 2,469

This mortality should be assessed in relation to the many accurate diagnoses obtained, but for a fuller appreciation it is necessary to examine the individual deaths.

Gillman's patient bled from an aberrant superficial artery in a "huge tuberculous liver," and that author considered that the patient could have been saved if surgery had been available. Both Sherlock's patients died from haemorrhage and had such severe liver damage that puncture only precipitated the inevitably fatal issue. One had acute necrosis, and the other subacute necrosis, general paralysis of the insane, and rectal carcinoma (Dible, McMichael, and Sherlock, 1943). The two patients of Roholm *et al.* (1942) died from haemorrhage, and although a haemorrhagic state existed vitamin K was not given. One had obstructive jaundice due to carcinoma of the head of the pancreas, and the other glandular metastases in the portal fissure. Volwiler and Jones's fatality followed puncture of the right main hepatic vein 4.5 cm. from the surface of a friable amyloid liver; intrahepatic bleeding led to gross splitting of the liver. Baron's patient had metastases in the liver and elsewhere and bled from a tear 1 cm. long; the bleeding and clotting times were not estimated.

Thus these seven deaths occurred where the prognosis was already hopeless, and three might have been avoided had the haemorrhagic state been treated.