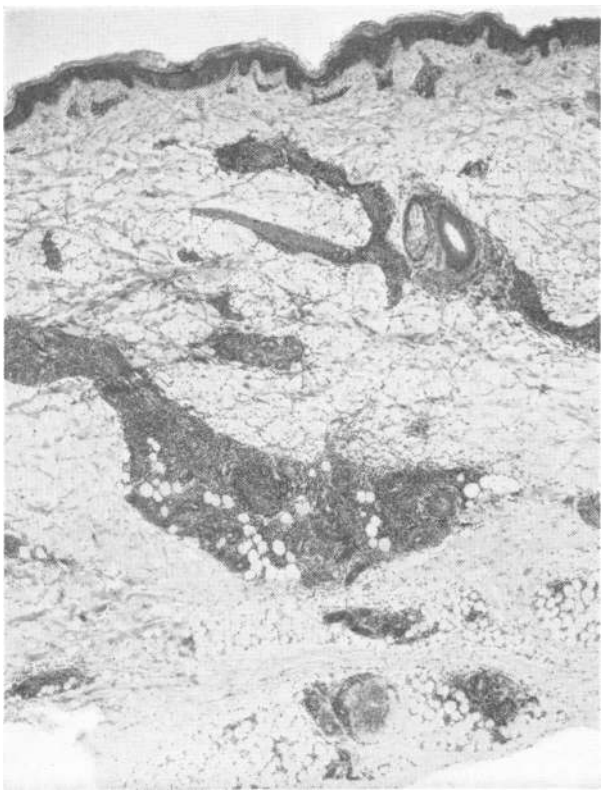

FIG. 1.—Skin from a tuberculin-sensitive human subject inoculated 48 hours previously with 10 units P.P.D. Hair follicles and sweat and sebaceous glands are embedded in a dense infiltrate of mononuclear cells of lymphocytic type. Similar cells are present also in the hypodermis and are abundant in the fat and around blood-vessels. (×35.)

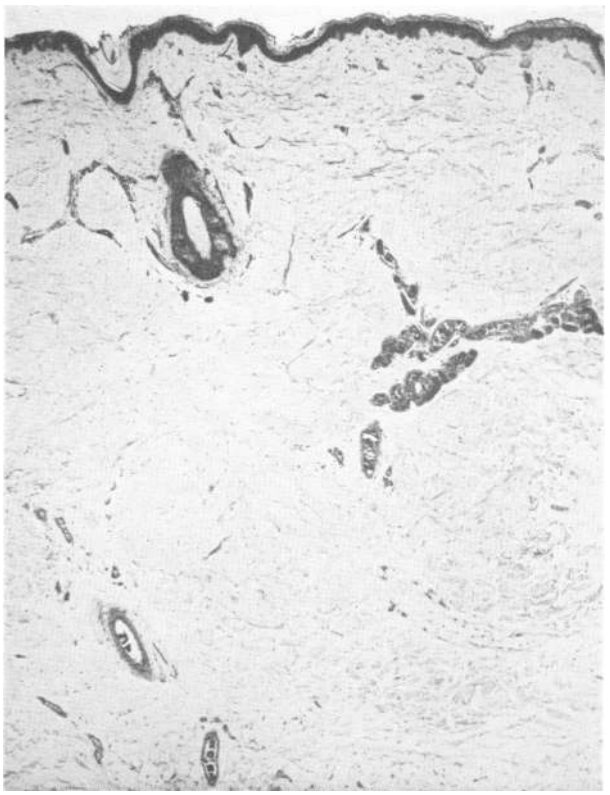

FIG. 2.—Skin from tuberculin-negative human subject inoculated 48 hours previously with 100 units P.P.D. The skin is essentially normal and shows the usual distribution of accessory skin structures and blood-vessels. (×30.)

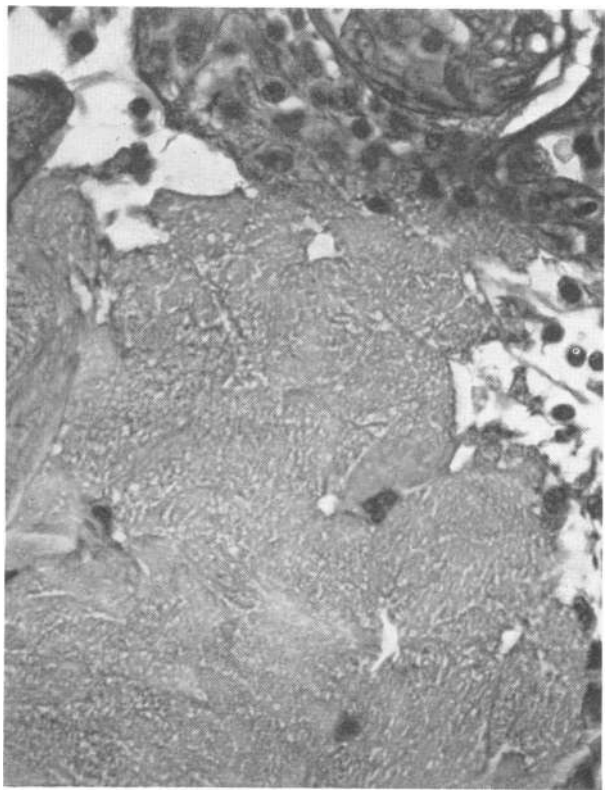

FIG. 3.—Area of connective tissue of the hypodermis close to a blood-vessel showing the loose reticular structure of the collagen in the normal uninhibited response. (×645.)

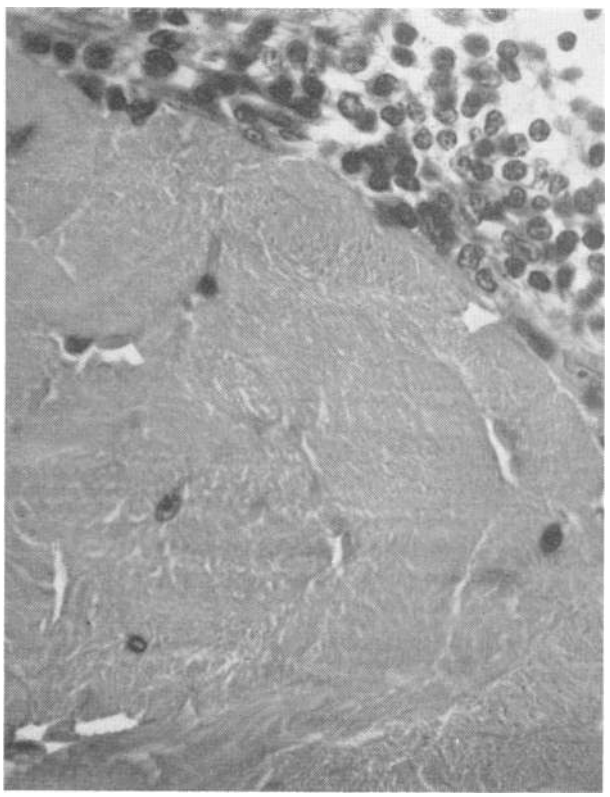

FIG. 4.—Area similar to that in Fig. 3 showing the compact character of the collagen in the inhibited response after treatment by D.S.U.H. (×645.)

INHIBITION OF MANTOUX REACTION BY DIRECT SUGGESTION UNDER HYPNOSIS

BY

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[WITH SPECIAL PLATE]

Experimental inhibition of immediate-type hypersensitivity responses by direct suggestion under hypnosis (D.S.U.H.) has already been reported (Black, 1963a, 1963b). The results are presented below of a study of the effect of D.S.U.H. on the delayed-type hypersensitivity response to tuberculin. Four Mantoux-positive subjects were tested by intracutaneous injection of purified protein derivative of tuberculin (P.P.D.) in one arm; the tests were then repeated in the other arm after a period of daily treatment by D.S.U.H. not to react to the injection. Records were made of the reactions in terms of the areas of firm swelling and erythema. Since the essential elements of the tuberculin reaction are characterized by cellular infiltration, full-thickness skin biopsy specimens were also taken for histological comparison.

Methods and Materials

Subjects.—Since skin biopsies were required, only male subjects were accepted, and because these investigations concerned a psycho-physiological phenomenon and not a therapeutic process the subjects were selected as being individuals thought likely to inhibit an allergic reaction following D.S.U.H., for reasons explained elsewhere (Black, 1963a). Twenty-eight male hypnotic subjects were screened by hypnosis and preliminary Mantoux-testing in the selection of the four found suitable for the experiment. Two of those chosen were deep-trance subjects, amnesic of the period of the hypnotic trance and capable of being psychologically regressed to childhood states, and two were medium-trance subjects, deeply hypnotizable, but neither amnesic nor regressable. A fifth Mantoux-negative medium-trance subject was also examined.

Experimental Procedure

The experiment was divided into six parts: (1) intradermal marking of the experimental areas; (2) preliminary assessment of tuberculin sensitivity; (3) clinical assessment, recording, and biopsy of the response area *before* D.S.U.H.; (4) treatment by D.S.U.H. not to react to P.P.D.; (5) clinical assessment, recording, and biopsy of the response area *after* D.S.U.H.; and (6) histological study of the results.

To limit any effects due to variations in technique each procedure was carried out by the same operator throughout the experiment: the inoculations with P.P.D. were given by Dr. T. S. L. Beswick, then of the Division of Immunological Products Control, M.R.C. Laboratories, Hampstead, London; the biopsy specimens were taken by Mr. I. F. K. Muir, consultant in the Mount Vernon Centre for Plastic Surgery, Northwood, Middlesex; and all other procedures were divided between us as indicated with initials in the text.

1. *Intradermal Marking of Experimental Areas.*—To facilitate histological examination of the biopsy material the skin of each subject was marked by intradermal inoculation with Pelikan black ink in volumes of 0.01 ml. at two sites on each forearm, 8 and 18 cm. below the level of the medial epicondyle of the humerus (S. B.). As with all inoculations throughout the experiment, the needle was inserted into the skin from distal to proximal, thus limiting the region of trauma involved to the distal portion of the specimen. After marking in this way, a period of not less than eight weeks was allowed in every case for any temporary effect of the ink to subside completely.

2. *Preliminary Assessment of Tuberculin Sensitivity.*—To facilitate comparison between one subject and another it was aimed to administer to each subject a quantity of P.P.D. sufficient to elicit a response with an area of firm swelling of diameter 1 cm. This degree of response was chosen as being a clear-cut positive of moderate intensity. The sensitivity of each subject was therefore determined three days before the first experimental test in each case. Into an area of the skin of the right forearm, 6 cm. lateral to the marked sites, intracutaneous injections were made of 0.1 ml. of P.P.D. solution diluted with Hanks's solution, equivalent to 10, 1, and 0.1 T.U. The responses at 48 hours were then recorded and that dilution selected which gave a response of the right order in each individual (S. B.). Throughout the experiment all areas of swelling and erythema were recorded by tracing directly on to "sellotape," and the areas were subsequently measured by planimetry, as already described (Black, 1963b).

3. *Clinical Assessment, Recording, and Biopsy of Response Area before D.S.U.H.*—Tests of the response before treatment by D.S.U.H. were carried out in each case at the distal marked site on the right arm; the proximal marked site on the same arm was used as a control area into which an inoculation of an equal volume of Hanks's solution was given. The intracutaneous inoculation with the selected dilution of P.P.D. at the distal site was given in a volume of 0.1 ml., using a 1-ml. tuberculin syringe and a No. 26 needle. The point of the needle was aimed to deliver the fluid in the skin just distal to the actual mark, so that the resulting bleb was in each case precisely under the mark. With a similar syringe and needle, 0.1 of Hanks's solution was then injected into the proximal site. At 48 hours an independent clinical assessment of the Mantoux reaction was made (J. H. H.) and the areas of weal and erythema were recorded (S. B.). Full-thickness skin biopsies were then taken from both sites under general anaesthesia. The biopsy areas were outlined so as to include 1 cm. of normal skin proximally and distally, the distal end being marked before incision with a silk suture tag to facilitate gentle handling. In addition 3 mm. of normal skin was included medial and lateral to the area of the response, so that the eventual specimen was a lozenge-shaped strip of skin approximately 3 cm. long and 1.6 cm. wide at its widest part.

At the proximal control site a strip of skin with similar dimensions was outlined around the intradermal mark and the distal end identified in the same way with a suture. Histological preparation was begun immediately after removal (J. S. F. N.).

4. *Treatment by D.S.U.H. Not to React to P.P.D.*—Forty-eight hours after the first biopsies were taken each subject started a 12-day course of daily treatment by D.S.U.H. in which forceful suggestions were given to the effect that there would be no reaction to any further inoculations with P.P.D. (S. B.). The subject lay on a couch and was hypnotized into a deep trance by use of a code word and a deepening technique already described (Black, 1963a). The principal suggestions were given in a standardized form with the words: "You are now different—you will no longer react to the injection as you did before: there will be no redness, no swelling, no heat, no itching, no pain—the skin will remain perfectly normal on both sites of your left arm after the next injections—your skin is now different, your left arm is now different, you are now different...." This was repeated no fewer than five times at each session together with any other suggestions judged by the hypnotist as likely to be effective according to the subject's personality and history. As additional reinforcement, both under hypnosis and in the waking state, all subjects were encouraged to relate any subjective experience associated with the suggestions and to discuss the experiment. Material obtained in this was then fed back to the individual concerned in the form of further suggestions. After five days each subject was also instructed that he would dream about the experiment, and the next day the dream material resulting from these suggestions was elicited under hypnosis and similarly used for reinforcement. Daily treatment in this way was continued until the biopsies were taken.

5. *Clinical Assessment, Recording, and Biopsy of Response Area after D.S.U.H.*—The second inoculation with P.P.D. was carried out while D.S.U.H. not to react was being given. The procedure was otherwise identical with procedure No. 3 in each case, except that the inoculations were given in the left arm. At 48 hours an independent clinical assessment of the Mantoux reaction was made (J. H. H.), the areas of swelling and erythema were recorded (S. B.), and full-thickness skin biopsies were taken and prepared for histological examination in the same way (J. S. F. N.).

6. *Histological Study of Results.*—Each specimen taken at operation was immediately pinned out in such a way as to prevent distortion during fixation, but also to allow free access of the fixative, which was a solution of saturated corrosive sublimate containing 4% glacial acetic acid. Cuts were later made transversely to the surface of a short axis so that penetration of the fixative would be as complete as possible. The wedges of tissue thus obtained consisted of (1) a central block within the limits of the marker spot area, 2.3 mm. in thickness; (2) an adjoining block containing the proximal zone of the marker spot, approximately 2.5–3 mm. in thickness; and (3) a distal adjoining block, which in this instance included, as well as the peripheral part of the marker spot, the site of the needle puncture. All these wedges of tissue were sectioned serially at 6μ in their entirety. The two remaining pieces of tissue, proximal and distal, were also cut transversely. These were then sectioned serially only to beyond the limits of pathological change. In the specimens taken from the areas of skin into which Hanks's solution alone had been injected, only the central blocks required to be cut serially in their entirety. The staining methods used included haematoxylin and eosin, orcein for elastic tissue, the periodic-acid Schiff procedure, and various trichrome methods for collagen and for fibrin.

Results

The results are discussed in terms of (1) assessment and measurement of the areas of swelling and erythema, (2) histology, and (3) subjective experience. The four experimental subjects are here designated A, B, C, and D; the control Mantoux-negative subject, E.

Assessment and Measurement of Areas of Swelling and Erythema

Before treatment all four subjects (A–D) gave definite positive tuberculin responses. The tuberculin-negative subject (E) gave no measurable response to 10 times the maximum amount of P.P.D. used in the other subjects. After treatment by D.S.U.H. not to react to P.P.D., the individual responses in A, B, and C were negative. A slight response was still evident in subject D, but was small enough to be described as Mantoux-negative. There was also no measurable swelling in subjects A, B, and C, and in D the area was reduced from 308 to 10 sq. mm. The area of erythema was reduced in all subjects but absent only in subject C. A summary of these results is given in the Table.

Mantoux Reaction: Effects of D.S.U.H. Not to React

Subjects: / Hypnotic trance state:	A Medium	B Deep	C Medium	D Deep	E Medium
Normal Response					
Dose (T.U.)	10	0·1	10	1·0	100
Clinical assessment	Pos.	Pos.	Pos.	Pos.	Neg.
Area of firm swelling (sq. mm.)	78	28	42	308	0
Area of erythema (sq. mm.)	289	154	218	770	0
Response after D.S.U.H. Not to React					
Dose (T.U.)	10	0·1	10	1·0	Not tested
Clinical assessment	Neg.	Neg.	Neg.	Neg.	
Area of firm swelling (sq. mm.)	0	0	0	10	
Area of erythema (sq. mm.)	48	83	0	319	

Histology

Despite the clear naked-eye differences between the normal uninhibited response and that following treatment by D.S.U.H. the cellular pattern was found to be indistinguishable in both instances. As would be expected in a 48-hour tuberculin response (Special Plate, Fig. 1), extensive lymphocytic infiltration in relation to blood-vessels was the outstanding feature, and many of the capillaries and venules were packed with lymphocytes. In the central zone, where the reactions were most intense, lymphocytes were also found in the connective tissue at some distance from the blood-vessels, either in small groups or in rows separating collagen bundles. They were also abundant in the interstices of the fatty tissue at the lower margins of the specimen. Polymorphs were scanty and confined mainly to capillary channels. A representative section from the Mantoux-negative subject (E) is shown in Fig. 2 (Special Plate).

The explanation of any difference between the responses before and after D.S.U.H. must therefore reside in non-cellular factors of the response, and of these the microscopically visible local oedema and

erythema are the most obviously relevant. Staining for fibrin was negative except in the normal uninhibited response of D, where there was a small haemorrhage; and here the fibrin was present only in the haemorrhagic area. However, it was noted consistently under standard conditions that the collagen in the control specimens and in the inhibited response stained more intensely than the collagen of the normal uninhibited response and that the fibril bundles themselves were more compact. With the periodic-acid Schiff procedure and trichrome methods used it was possible to define in the normal uninhibited response a distinct separation between the fibrillar material and ground substance in the collagen (Special Plate, Fig. 3). In the control material (no P.P.D.) and in the inhibited response (Special Plate, Fig. 4) the individual fibrils were not separated by obvious interfibrillar material in this way. It must be assumed, therefore, that this appearance is due to the accumulation of fluid between the fibrillar components of the collagen.

Distinction was less easy in respect of the erythema. At the microscopical level it was not possible on structural findings to divide the material into two groups as was the case when using collagen appearance as a method of differentiation. Beyond the area of lymphocytic infiltration the capillaries in the third plexus just below the epidermis regularly appeared more conspicuous and congested than in the controls, but areas could also be found in the inhibited series in which the dilatation and congestion were of the same order.

Subjective Experience

All subjects reported paraesthesiae associated with the left arm after the sessions when treatment was given by D.S.U.H. not to react to P.P.D. As stated, the inoculations with P.P.D. were given under hypnosis, and all four subjects reported afterwards that they were unaware of the injection, although no specific instructions concerning anaesthesia had been given. When seen at 24 hours for further treatment by D.S.U.H. all subjects reported that there was " no reaction " and that they had felt no itching, no pain, and no subjective evidence of inflammation of any kind. At 48 hours, when subjects A and B showed small areas of erythema and subject D a very small area of swelling as well, all four still maintained that they felt " nothing like the reaction before."

Discussion

The experiments described above were designed so far as possible to exclude any variations in the intensity of the successive tuberculin reactions in a given subject apart from such as might be due to hypnotic suggestion. Thus the subjects were well sensitized to tuberculin, and a small test dose was sufficient to elicit a reaction which was quite definite, though of only weak-to-moderate intensity. It is unlikely that either the preliminary injections or the tests themselves would have affected the subsequent reactions; in so far as any desensitization might have occurred it would have been most likely to affect the first test, which was that carried out before any suggestion under hypnosis had been made. The solutions were prepared and the intracutaneous injections were performed by persons not connected with the investigation, and the subsequent assessments of the macroscopic responses were made independently by two observers.

Although the findings relate to only four test subjects they indicate that there was a clear-cut diminution in the macroscopic response to tuberculin after D.S.U.H. not to react. This was evident in the size of the central firm swelling and of the surrounding area of erythema. Despite the absence of any obvious swelling in three of the subjects a palpable area of induration was present at the sites injected with tuberculin.

When examined histologically after 48 hours all the reaction sites showed extensive mononuclear-cell infiltration typical of a tuberculin response; such changes were absent from control sites injected with diluent alone, or from sites in a Mantoux-negative control subject injected with much larger amounts of tuberculin. Thus, although the macroscopic reactions were not intense, the underlying cellular changes indicated that an extensive specific reaction had occurred. There were *no* detectable differences in the degree of mononuclear-cell infiltration in reactions elicited before and after treatment by D.S.U.H., and in this respect the reactions were not affected by the treatment. However, the naked-eye differences were confirmed at the histological level by the observation that the fibrillar components of the collagen bundles, as compared with those of normal skin, were conspicuously separated from one another in the reactions studied before treatment with D.S.U.H., whereas after treatment this feature was virtually absent. Even in subject D, in whom a slight degree of firm swelling was recorded, specimens taken before and after D.S.U.H. could be readily distinguished. The detailed histological observations will form the subject of a separate publication by one of us (J. S. F. N.).

Tuberculin-reactive humans almost invariably have circulating antibodies against components of the tubercle bacillus, especially against carbohydrate constituents, and traces of the latter are found in nearly all preparations of tuberculin, including P.P.D. (see, for example, Long, 1958). Consequently tuberculin reactions carried out in man almost inevitably show, in addition to the mononuclear-cell infiltration typically associated with a delayed-type response, characteristics associated with Arthus-type hypersensitivity. These, in experimental animals, are represented by vascular damage, oedema, and a marked degree of polymorphonuclear migration; they are maximal after 6 to 18 hours, but may persist for 48 hours, although by then the polymorphonuclear leucocytes will largely have become pyknotic or will have been destroyed. It is difficult to be certain whether the oedema seen in the human tuberculin reaction represents the residue of an Arthus-type response or is truly associated with the delayed-type response. Nevertheless our findings suggest that it was the exudation of fluid in the response that was affected by D.S.U.H. not to react, while the cellular infiltration characteristic of the delayed-type response was essentially unchanged. This evidence would therefore seem to indicate that the mechanism of inhibition by D.S.U.H. involves a vascular constituent and that the process is probably similar to the inhibition by D.S.U.H. of the immediate-type hypersensitivity weal and erythema response already reported (Black, 1963a, 1963b).

Conclusions

It is concluded that the tuberculin reaction as observed clinically in the Mantoux test can be inhibited by D.S.U.H. in suitable subjects, although histologically there may be no observable change in the degree of cellular infiltration. In the normal uninhibited response,

however, the connective tissue was demonstrated to be consistently less compact than in the inhibited response and control material, and this is assumed to be due to an accumulation of fluid between the fibrillar components of the collagen. On this evidence it is thus further concluded that such inhibition of the Mantoux reaction by D.S.U.H. probably involves control of the fluid exudation normally present and that therefore in the mechanism of inhibition a vascular constituent is likely to be involved.

Summary

The effect of direct suggestion under hypnosis (D.S.U.H.) on the delayed-type hypersensitivity response to tuberculin in the Mantoux test was studied in four hypnotic subjects and full-thickness skin biopsies were taken for histological examination. The results showed that the tuberculin reaction as observed clinically in the Mantoux test was inhibited by D.S.U.H., and that while histologically there was no observable change in the degree of cellular infiltration, there was evidence that the exudation of fluid had been inhibited. It is concluded that the Mantoux-positive reaction can be inhibited by D.S.U.H. to give a Mantoux-negative result and that a vascular constituent of the reaction is probably involved in the mechanism of inhibition.

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ENCEPHALOPATHY AFTER PORTACAVAL SHUNT

BY

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[WITH SPECIAL PLATE]

Portacaval shunt can be a life-saving surgical procedure in portal hypertension, but it exposes the patient to a risk of encephalopathy. The risk is not inconsiderable. Walker, Shaldon, and Vowles (1961) reported the cases of five patients who were severely incapacitated by portal-systemic encephalopathy out of 35 who survived the operation for more than five years. Read, Laidlaw, and Sherlock (1961) were able to demonstrate some cerebral effect in the majority of 21 patients with portal hypertension operated on in this way and followed for shorter periods.

The varied symptoms of portacaval encephalopathy are becoming widely known, but reports of the structural changes in the nervous system are still few. Adams and Foley (1953) described the neuropathological findings in hepatic coma of varied origin and reviewed the literature; but in encephalopathy of the present type, deviation of portal blood from the liver can be identified as the chief determinant cause. Encephalopathy may result from portacaval anastomoses even when hepatic damage is slight or absent, as in the case reported by Baltzan, Olszewski, and Zervas (1957). The most striking lesion reported by these authors was status spongiosus of the cerebral cortex and to a less extent of the basal nuclei, findings identical with those reported here. Spongy change seems to occur more often in long-standing portacaval encephalopathy than in hepatic encephalopathy due to most other causes, but in Wilson's disease lesions of this kind are well known.

Case History

The patient was aged 61 when he died in 1962. He gave a history of jaundice in 1943. Nine years later several haematemeses occurred, and an end-to-side portacaval anastomosis was performed in February, 1953, by Professor H. W. Rodgers, who found the liver nodular and 70% of normal size; the spleen was twice normal size; the portal-venous pressure was 240 mm. of saline. The only biochemical derangement of liver function detected was in the bromsulphthalein test; pre-operatively 3% was retained after 45 minutes, but after operation the figure was 17%. Recurrent attacks of mental confusion marked the first two post-operative years. The early stages of this portacaval encephalopathy and the patient's initially good response to a reduction in his protein intake have been described by Hurwitz and Allison (1955). Copper studies carried out in November, 1955, have been reported and discussed by Warnock and Neill (1958, Case 3); a moderate depression of serum copper, caeruloplasmin, and copper oxidase were detected. The levels were higher than those found in Wilson's disease, and the abnormalities were thought to be the result of the liver disease. An amino-aciduria was also noted. Kayser–Fleischer rings were not present. A total of 2.4 g. of dimercaprol was given over three days in 1955 by intramuscular injection. Two courses of calcium versenate by mouth were given, in February and in May, 1957—to a total of 12 g. in each course.

The patient was admitted on many occasions to Claremont Street Hospital under Dr. Allison's care. By March, 1956, bromsulphthalein retention had increased to 37%, and by January, 1959, serum albumin had fallen to 2.3 g. and globulin had risen to 3.3 g./100 ml. Varices were still present in the oesophagus and occasional small haematemeses occurred; the haemoglobin was maintained for some time without difficulty. The patient gradually became weaker, paler, thinner, and more tremulous. His final hospital admission lasted nearly two years and was marked by mental impairment of fluctuating severity with choreiform and athetoid movements of mouth, head, and upper extremities. At times he was almost inarticulate and even semicomatose. Reduction in protein intake now failed to alleviate his symptoms with regularity. On other occasions his speech was better and he was able to sit up in a chair, darn socks, and engage fussily in small activities.

Throughout 1961 his general condition deteriorated. He vomited dark blood and coffee-grounds material from time to time, and blood transfusion was required twice. In July he was lightly jaundiced for a week or two; in August the serum albumin was 2.6 and globulin 3.5 g./100 ml. Signs of large-bowel obstruction were noted intermittently and an abdominal mass, first detected in August, 1957, enlarged and became readily palpable, mimicking a large spleen. Abdominal veins were distended. On *x*-ray examination a focus of calcareous material was seen over the sacrum. Fluid was not detected in the abdomen, but oedema was present in the legs. His mental and physical condition deteriorated further and he died on February 3, 1962.

Necropsy Findings

Necropsy was carried out two hours after death and is summarized as follows. Atrophic post-necrotic cirrhosis (Special Plate, Fig. 1), long-standing surgical portacaval