TRANSFUSION REACTIONS AND FATALITIES CONSEQUENT ON CIRCULATORY OVERLOADING*

BY

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Much research has recently been devoted to the identification of abnormal agglutinogens and iso-agglutinins. Noteworthy has been the discovery of the Rh factor by Landsteiner and Wiener (1940). Attention, too, has been focused on the grave danger of transfusion of incompatible blood. It is well known that death may follow transfusion of an incompatible agglutinin—e.g., B cells into an A recipient, etc. Yet recovery may follow such a mishap. Kilduffe and DeBailey (1942) give the mortality rate as about 50%. The total incidence of haemolytic reactions due to transfusion of incompatible cells over a large series of cases is very low. Thus Kilduffe and DeBailey in a collected series of 40,000 transfusions found 80 (0.18%) haemolytic shock reactions, of which 32 (0.07%) were fatal. The total number of deaths was 45. I have personally seen only two such cases in 10 years; both were fatal, and both were due to faulty blood grouping by the slide technique. However, as Wiener (1939) remarks, "it is fortunate that the transfusion of incompatible blood is not always fatal; indeed, in a number of instances the reactions were so mild or insidious that they passed unnoticed or were attributed to other causes." Serious reactions have followed transfusion of as small a quantity as 10 c.cm. of incompatible blood. In some cases death has been almost instantaneous.

According to Kilduffe and DeBailey, who cite Bordley, there is a definite relation between the amount injected and the mortality. Recoveries have been reported—e.g., two cases by Wiener *et al.* (1941)—and one of these was a Group O recipient who received Group B cells. A similar case was reported by Mollison and Young (1941).

Further, Wiener noted that second in importance to injection of blood of a wrong group as a cause of severe or fatal reactions to blood transfusions is the use of universal donor blood. He mentions that, according to Gesse (1935), the use of universal donor whole blood on recipients of groups other than O is apt to be followed by a haemolytic reaction if more than 200 c.cm. is transfused when the patient is suffering from severe anaemia, or if the serum of the donor is of high titre for the recipient's cells. However, while this is probably true of severely anaemic recipients of the A-B system, it is certainly not true of the ordinary run of casualties. The use of universal donor blood in recipients of all groups suffering from acute haemorrhage has been repeatedly proved to be safe and successful in thousands of casualties in the present war.

A Frequent Cause of Reactions

Due regard having been paid to deaths consequent upon agglutination and haemolysis either of donor's or of recipient's cells, there still remains in my opinion a more frequent cause

of reactions and fatalities. I refer to overloading of the circulation in the chronically anaemic and diseased subject.

In the Welsh Board of Health Regional Transfusion Service there is a mutual arrangement whereby all transfusions given in hospitals are notified on a standard record form. With the aid of these records it has been possible to track down and investigate some interesting transfusion reactions. It is noteworthy that in 2,300 transfusions notified in the last 2½ years there has not been a single instance of death due to transfusion of cells of an incompatible blood group. There have been some cases of death from pulmonary oedema and cardiac failure due to circulatory overloading in chronically anaemic subjects; also, there have been a few haemolytic reactions—e.g., jaundice or haemoglobinuria—following the use of Group O or of homologous blood.

Whitby (1941), in an address to the Shaftesbury Military Hospital Medical Society on transfusion for battle casualties, noted that these subjects contrasted with those commonly requiring transfusion in peacetime practice in that they had been previously healthy and had no cardiac disability; it was therefore safe to give at least two pints of blood or plasma quickly to anyone with a blood pressure consistently below 100 mm. Hg, and then to carry on with a slower drip transfusion. It is common knowledge that one problem which sometimes confronts a resuscitation officer when dealing with air-raid casualties is how to get the fluid in quickly enough. Indeed, positive pressure may have to be used, and it may be necessary to introduce a pint of plasma or blood in 15 minutes.

The reason why no cardiac embarrassment occurs in Service casualties is, of course, simple. First, the introduced fluid replaces lost fluid. Secondly, the myocardium is invariably normal and therefore functions efficiently. It is well known in clinical medicine that it is impossible to strain a normal heart. This has been abundantly proved experimentally. I quote here from Starling (1930):

"Experimentally it is found that the isolated heart has a marvellous power of adaptation—i.e., regulating its activity according to the mechanical demands which are made upon it. Thus if we keep the venous inflow constant it makes no difference to the output of the heart whether the average arterial pressure be maintained at 80 or 160 mm. Hg, although in the latter case the heart must do twice as much work in order to keep the outflow at the same level. Again, if we maintain the arterial pressure constant and alter the venous inflow, we find that within very wide limits the heart is able to expel against the arterial resistance the whole of the blood which flows into it from the veins. In this way we can alter the output of a small heart weighing 50 grammes from 300 to 3,000 c.cm. per minute."

Further, Hayward (1942) showed that even in only reasonably healthy subjects serum or plasma could be introduced fairly rapidly. He transfused 50 subjects suffering from chronic arterial disease each with 800 c.cm. of pooled serum, the fluid being introduced at a rate of 5 to 15 c.cm. a minute. The incidence of rigors was 30%. If the serum was injected slowly the cardiovascular system adjusted itself to the increased blood volume readily, there being no increase of venous pressure, and the blood pressure in most cases showed no significant change.

* A paper read at a meeting of Regional Transfusion Officers held at Leeds on March 4, 1943.

Overloading after Rapid I.V. Injections

Sharpey-Schafer and Wallace (1942) investigated the question of circulatory overloading following rapid intravenous injections. They used subjects without cardiovascular disease, and up to 2,000 c.cm. of saline, serum, or blood was injected at rates of from 54 c.cm. to 168 c.cm. a minute. The venous pressure was raised up to 11 cm. H₂O when there was considerable retention of injected fluid in the circulation, as indicated by the fall in the Hb. Radiographs showed an increase in the diastolic size of the heart, enlargement of pulmonary arteries, and prominence of the vascular markings in the lung fields. Vital capacity was diminished, but there was no pulmonary oedema. Despite rise of venous pressure many subjects had no increase in heart rate. However, 4 out of 12 had electrocardiographic signs of heart stress. There were no important symptoms. Those authors stated: "There is evidence that the peripheral and pulmonary capillaries and veins dilate to accommodate the increased blood volume." Lastly, Kilduffe and DeBailey mention that they have infused 650 c.cm. of blood in three minutes! They point out that when myocardial disease is present transfusions must be given with caution; but, apart from this, the suggestion of the danger of embarrassing and overloading the cardiovascular system by rapid transfusion has no rational basis, and they therefore rightly condemn the term "speed shock."

It is apparent, therefore, that, if the myocardium is normal and is functioning efficiently, there is nothing to be feared from rapid transfusion or from overloading the circulation.

Risks of Transfusion in Anaemia

The position is, however, quite otherwise when the functional efficiency of the myocardium has been affected by long-standing anaemia or severe toxæmia. Very grave risk indeed may attend the rapid transfusion of the chronically ill and severely anaemic subject. The risk seems to be particularly grave in pernicious or aplastic anaemia, in severe anaemia consequent on chronic and oft-repeated haemorrhage—e.g., of uterine or gastric origin—in malignant disease associated with cachexia and haemorrhage, and in puerperal cases associated with the effects not only of much haemorrhage but also of sepsis.

Plummer (1936) reported five deaths from heart failure after transfusion in chronically anaemic subjects. Careful preliminary tests were performed and there was no incompatibility. Death occurred, often with pulmonary oedema but without evidence of haemolysis, from 30 minutes to 19 hours after transfusion. It was noted that few such cases had been reported; but this was not a true index of their incidence, for these cases were seen in a two-and-a-half-year period at one hospital. They were typical examples of the danger of rapid transfusion or circulatory overloading in subjects with enfeebled cardiac action due to long-standing anaemia. One case well illustrated the very grave danger of transfusion when circulatory failure was already present.

According to Riddell (1939) the commonest cause of a fatality following blood transfusion is circulatory failure. It may be due to overloading of the circulation or it may be secondary to a rigor. Whitby (1942) has pointed out that "too fast a rate can cause a rigor, which ceases instantly if the rate is slowed." As Riddell rightly contends, if the blood volume is normal—i.e., not depleted by haemorrhage—the production or not of circulatory failure after the intravenous infusion of fluid will depend on three factors—namely, the volume of blood introduced, the rate of its introduction, and the mechanical efficiency of the myocardium at the time. Marriott and Kekwick (1940), in their admirable paper on rate and volume of transfusion of blood in anaemia, laid down four important cardinal principles for the transfusion of severely anaemic subjects. These are: (i) rate not to exceed 1 c.cm. per lb. of body weight per hour; (ii) if the Hb is 25% or less, and if there is cachexia or cardiac or respiratory disease, the rate should not exceed 0.5 c.cm. per lb. of body weight per hour; (iii) maintain steadily the determined rate of transfusion and do not accelerate for short periods; (iv) watch carefully for signs of heart failure (dyspnoea, cough, basal rales) and suspend transfusion if they appear. They

consider that the giving of a pint in half an hour is irrational and unsatisfactory. All too often these important principles are neglected.

Too Rapid Transfusion in the Anaemic

The following cases illustrate the dangers of too rapid transfusion in the severely anaemic and chronically ill subject.

Case 1.—A man aged 54 was admitted to hospital suffering from chronic anaemia and cachexia due to carcinoma of the stomach. He weighed about 10 st. and had lost some 3 st. in weight. The Hb was 50% and the blood group was O. Transfusion of 10 oz. of Group O whole blood was given in 30 minutes. He then experienced severe pain in the chest and headache. He had a moderate rigor, which lasted about half an hour, and was left with a feeling of tightness in the chest. The transfusion was stopped. Next day he still complained of tightness in the chest, and rhonchi were audible in both lungs. He recovered. Careful cross-matching tests after the transfusion showed no incompatibility. The patient died 16 days later of unrelated causes. There was no necropsy.

Comment.—If one works on the principles laid down by Marriott and Kekwick—i.e., 1 c.cm. per lb. of body weight per hour—this patient should have received 140 c.cm. in an hour, or 70 c.cm. in half an hour. In fact, he received 280 c.cm. in half an hour—i.e., the transfusion was given at four times the rate it should have been given.

Case 2.—A multipara aged 39 was admitted urgently for treatment of profound secondary anaemia. The patient stated that she had had a miscarriage, had advanced in pregnancy seven months, and that she had been bleeding per vaginam for 19 days. However, her doctor subsequently affirmed that, so far as he could ascertain, bleeding had gone on intermittently for several weeks. The blood pressure was 150/80 on admission, the Hb 25%, and the blood group A. The next afternoon a transfusion of Group A whole blood was given. Full cross-matching tests were carried out by an experienced pathologist and there was no incompatibility. The patient had not previously had a transfusion. Four oz. was infused in 10 minutes, when the patient complained of a sense of constriction in the chest, severe precordial pain, and a sense of coldness, which in a minute or so was followed by a rigor lasting a few minutes. Slight cyanosis of the lips was noted. The transfusion was discontinued. An hour later her condition had deteriorated very much: she was semi-comatose, had slight cyanosis of the lips, and the pulse was rapid and feeble. Some 2½ hours later the condition seemed to have improved, and it was decided to try the effect of a further transfusion. A second bottle of Group A blood was used. Cross-matching tests showed complete compatibility. In the next two hours a further 8 oz. of blood was given. Some 4½ hours after starting the first transfusion the patient died of circulatory failure. *Necropsy.*—Weight of body about 10 st.; nourishment good. There was profound anaemia of all organs except the lungs and liver, which were congested. The lung bases were oedematous; the kidneys normal. The uterus was 5 in. long and contained placental fragments. There was no evidence of intravascular haemolysis.

Comment.—In this case the rate of transfusion was too rapid. The determined rate of transfusion, since the Hb was only 25%, should have been 0.5 c.cm. per lb. of body weight per hour. Thus from the first bottle 114 c.cm. was given in 10 minutes, whereas the rate should have been 70 c.cm. in 60 minutes. The rate of introduction of the blood was about 11 c.cm. a minute, whereas it should have been about 1.5 c.cm. a minute.

Case 3.—A 36-year-old woman had suffered from symptoms of dyspepsia and epigastric pain after meals for two years. Symptoms had become worse in the last four months. Three days before admission she had a very severe haematemesis. It is not known whether she had suffered from melaena or haematemesis previous to this. She was sent into hospital urgently and was found to be profoundly anaemic and weak. The blood count showed 1,600,000 red cells and 22% Hb. She was at once given a transfusion of stored Group O whole blood. The recipient's group is not known, but a cross-matching test between the recipient's serum and the donor's cells showed compatibility. In barely 30 minutes the patient was given 540 c.cm. of blood. Transfusion had just ended when she had a rigor. Her general condition soon deteriorated, respirations became faint and rapid, and 1½ hours after the cessation of transfusion she was comatose. After a further 5 hours the patient was still comatose and had what seemed to be a slight convulsion. Death occurred 5½ hours after the end of transfusion. There was no necropsy.

Comment.—The weight of this patient was estimated to be about 9 st. If this is so, and with a Hb of 22%, the rate of

transfusion should have been 0.5 c.cm. per lb. of body weight per hour—i.e., about 70 c.cm. in one hour instead of 540 c.cm. in half an hour.

Cases of Too Large a Transfusion

The next two cases concern the question of overloading the circulation by the factor of transfusion of too great a volume of fluid.

Case 4.—A woman aged 24 was suffering from severe chronic anaemia (pernicious ?), albuminuria, and oedema of pregnancy. The haemoglobin was 22%. A radiograph revealed a much-dilated heart (see Fig.). A transfusion of concentrated red cells,* from

Radiograph showing the severe cardiac dilatation in Case 4.

2 pints of whole blood, was given with especial care at a very slow drip rate after careful cross-matching. Both donor and recipient belonged to Group A. After six hours about 400 c.cm. had been given when the patient complained of a sensation of constriction in the chest and developed a slight irritative cough. The ward sister had been warned to look out for these symptoms, and the transfusion was promptly stopped. The symptoms immediately ceased and there was no ill effect afterwards. The recipient, who was Rh-positive, was much improved.

Comment.—This case supports the opinion that in cases of severe chronic anaemia it is better to give initially repeated small transfusions of about 250 c.cm. than single large-volume transfusions. I think, too, that concentrated red cells should be used in preference to whole blood, so that the maximum number of cells may be given in the minimum volume of fluid.

Case 5.—A man aged 40 was admitted to hospital suffering from severe injuries to the left leg and right ankle. It is not evident that there had been extensive loss of blood. The patient was somewhat shocked. No regular blood-pressure recordings were made, and in the space of two or three hours he received 5 pints of Group O whole blood. The blood group of the recipient is not known. All the donors' blood had been carefully checked and grouped on both agglutininogen and agglutinins by the issuing blood store. It is stated that the transfusion was persevered with because the pulse rate continued to rise. The left leg was amputated above the knee a couple of hours later. The patient died shortly after operation. *Necropsy.*—The body was well nourished and showed rigor mortis. The right auricle of the heart was tremendously distended and ballooned. So great was this distension that the heart-muscle fibres were separated from each other, rendering the wall of the right auricle semi-transparent. The right auricular appendage was grossly distended. A small agonal thrombus was present in the main branch of the right pulmonary artery. The lungs were extremely oedematous, and there was a little aspirated food in the bronchi. The endothelium of arteries and veins showed intense haemoglobin staining. *Histology.*—Right kidney: There was autolysis of the kidney parenchyma. In one area a very small amount of yellowish pigment was seen in the tubules of the medulla. Left kidney: Some of the vessels showed lysis of red corpuscles and the presence of numerous bacilli. In other vessels there was much brown pigment consisting of coarse granules arranged in clumps. The lungs showed oedema, and the blood in all vessels appeared to be haemolysed. There was fat embolism of the lungs, but of only moderate degree.

* All the plasma was siphoned off and half its volume replaced with citrate solution.

Comment.—This case illustrates well the grave dangers of uncontrolled resuscitation—i.e., no careful blood-pressure recordings, etc. There can be no doubt that too great a volume of blood was transfused. The essential cause of heart failure was circulatory overloading due to the introduction of too great a volume of blood. The haemolysis was shown to be a post-mortem phenomenon.

Indiscriminate use of universal donor blood is to be deprecated. Compatibility tests ought always to be performed in routine hospital practice, and homologous blood given if it is desired to raise the haemoglobin level. If facilities for tests are not available, then the services of the nearest blood depot should be utilized. In emergencies—e.g., wound shock and haemorrhage—it is safer and wiser to use serum or plasma in order to achieve the object in view—namely, restoration of blood volume—and in the interim full cross-matching tests may be carried out should whole blood be necessary.

Discussion

A case very similar to that just quoted was described by Tubbs (1942). His patient, a 46-year-old man, suffered from chronic lung abscess. During lobectomy there was moderate blood loss. One pint of saline and 3½ pints of blood were given. After operation his condition was excellent, but on being returned to bed he became cyanosed, pulmonary oedema set in, and thin watery blood-stained fluid accumulated in the air passages. Death occurred two hours after operation. At necropsy the right side of the heart was bloated with blood, and the lungs and air passages were filled with great quantities of blood-tinged oedema fluid. As Tubbs points out, the myocardium of this man was not normal, but had been subjected to the chronic toxic effects of a lung abscess for 12 months. Under such conditions the danger of rapid intravenous injections was only too real. Incidentally A. S. and H. G. Grünbaum (1911), in a forthright article on "post-operative drowning," long ago warned of the grave danger of post-infusional death after intravenous saline injections in cases of surgical collapse. It is evident that this warning needs repetition at the present time.

While the most important cause of transfusion reactions or fatalities is the injection of incompatible blood, it nevertheless seems certain that a common cause of such mishaps is overloading of the circulation. I believe that this remark applies especially when universal donor whole blood is used on anaemic recipients of the A-B system. Not only is there grave danger in overloading the circulation in such cases, but if in addition to this a reaction occurs between the donor's agglutinins and the recipient's cells, then a condition of "haemolytic shock" may be set up. This condition of haemolytic shock in association with a sudden overloading of the circulation from too rapid transfusion may well prove too great a strain on the weakened anaemic myocardium, and circulatory failure may ensue, with fatal result. It should be borne in mind that in severe chronic anaemia the functional capacity of the heart may be seriously impaired and its powers of reserve negligible. The cavities are often much dilated, and the capacity to undergo further compensatory dilatation may be so grossly diminished that the heart becomes unable to accommodate a given volume of blood if too rapidly introduced. Starling has pointed out that an increased diastolic volume means greater length of the muscle fibres of the heart wall. As with voluntary muscle fibres, the energy of contraction is a function of the length of the muscle fibres—a generalization known as "Starling's law of the heart." But in the chronically anaemic myocardium dilatation may be present, possibly of considerable degree (see Fig.). Clearly, under such conditions the heart fibres, already stretched and not contracting to their fullest extent, have little capacity to stretch further. Consequently the right auricle becomes unable to accommodate a large volume of blood suddenly introduced, and fails to expel its contents fully. Failure then ensues. The extra volume of blood must become accommodated somewhere, and, as Sharpey-Schafer has shown, this is done in the vessels of the lungs. Hence the earliest signs of overloading are crepitations due to pulmonary oedema. The precordial pain of which these patients so often complain is presumably due to overdistension of the right auricle and tension on the

pericardial sac. At necropsy fatal cases constantly show cardiac dilatation and pulmonary oedema and congestion. The pulmonary oedema is evidence of left-sided heart failure.

Treatment should comprise prompt cessation of transfusion, the administration of oxygen, and the injection of morphine and atropine. Adrenaline is contraindicated. Venesection may be necessary to relieve the load on the right heart.

A reasonable rate of transfusion in severe chronic anaemias is 40 drops a minute, and the giving of one pint of whole blood should be spread over at least three hours if disasters from circulatory overloading are to be avoided.

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REFERENCES

- Gesse, E. R. (1935). *Dtsch. Z. Chir.*, **245**, 371.
 Grünbaum, A. S. and H. G. (1911). *British Medical Journal*, **2**, 1281.
 Hayward, G. W. (1942). *Ibid.*, **1**, 285.
 Kilduffe, R. A., and DeBakey, M. (1942). *Blood Bank and Technique and Therapeutics of Transfusions*, St. Louis.
 Landsteiner, K., and Wiener, A. S. (1940). *Proc. Soc. exp. Biol.*, N.Y., **43**, 223.
 Marriott, H. L., and Kekwick, A. (1940). *British Medical Journal*, **1**, 1043.
 Mollison, P. L., and Young, L. M. (1941). *Lancet*, **2**, 635.
 Plummer, N. S. (1936). *British Medical Journal*, **2**, 1186.
 Riddell, V. H. (1939). *Blood Transfusion*, Oxf. Univ. Press, London.
 Sharpey-Schafer, E. P., and Wallace, J. (1942). *British Medical Journal*, **2**, 304.
 Starling, E. H. (1930). *Principles of Physiology*, London.
 Tubbs, O. S. (1942). *British Medical Journal*, **2**, 406.
 Whitby, L. E. H. (1941). *Ibid.*, **1**, 533.
 — (1942). *Lancet*, **1**, 581.
 Wiener, A. S. (1939). *Blood Groups and Blood Transfusion*, Springfield.
 — et al. (1941). *Amer. J. clin. Path.*, **11**, 102.

EFFECT ON THE UTERUS OF EXTRACTS OF GORSE (ULEX GALLII)

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In parts of Yorkshire and Derbyshire infusion of gorse is a traditional remedy for retained placenta in the cow. An apparently successful demonstration of its efficacy aroused our curiosity and led us to test its effect upon isolated strips of guinea-pig uterus. Sustained tonic contraction was consistently produced by addition of the watery extract to the fluid in which the uterus was suspended. The consequent investigation was designed to isolate the active principle and to determine its chief pharmacological actions. These objects have not yet been achieved, but we think that the results obtained so far should be published.

Preparation of Extracts

Terminal shoots of gorse from several bushes were passed through a farm hay-chopper and then through a hand tissue-grinder to give a coarse powder. Aqueous extracts, prepared by boiling the minced gorse in water, N/100 HCl, or phosphate buffer solution pH 8.4, were found to be of approximately equal potency when tested on isolated guinea-pig uterus, whereas direct extraction of the gorse with a number of organic solvents failed to yield active products. During attempts to isolate an active principle from the infusions many potent fractions of varying degrees of purity were obtained, but inexplicable irregularities decided us to carry out pharmacological tests on a stock pool of concentrated extracts prepared by the following method, which gives consistently reproducible results and eliminates a considerable amount of inert material.

250 g. of minced tissue are boiled gently for half an hour in 2.5 litres of distilled water. The infusion is strained through muslin and then filtered through a layer of kieselguhr. Approximately 1,800 ml. of clear greenish-brown filtrate with a characteristic odour and acid to methyl red are obtained. Saturated solution of basic lead acetate is added until there is no further precipitation. The

voluminous precipitate is filtered off through kieselguhr and discarded. The filtrate, after removal of lead as lead sulphide, is reduced to about 1/50 volume by distillation at 60–65° C. under reduced pressure. To this concentrate 9 volumes of absolute alcohol are added. A flocculent precipitate forms almost immediately, which is filtered off and discarded. The alcohol is removed from the filtrate by distillation at 60–65° C. under reduced pressure; this leaves a brown viscous fluid together with gummy material adherent to the sides of the distillation flask. The gummy material goes readily into solution in distilled water, and the flask washings are used to make up the total volume to exactly 1/50 volume of the original infusion filtrate.

Several batches of gorse extracted in this way yielded concentrates of indistinguishable potency; they were therefore pooled to give the stock concentrate referred to hereafter as "S."

The concentrate, when freshly prepared and still warm, has a characteristic pungent odour which is lost to some extent on storage. It is a clear golden-brown viscous fluid and is acid to methyl red. Alkalinization with NaOH causes a deepening of the colour.

Pharmacological Actions

Technique.—In all tests on isolated tissues duplicate preparations were suspended in either Ringer's or Van Dyke's solution, the capacity of each bath being 20 ml. One-ml. quantities of various dilutions of neutralized extract were used, and the concentrations recorded were calculated on the assumption that a further dilution of 1 in 20 was effected by mixture with the bath fluid. The usual period of observation was two minutes, but effects over longer periods were also frequently studied. The tests on intact cats were made under nembutal anaesthesia, the uterus being kept submerged in Ringer's solution in the abdominal cavity.

Isolated Guinea-pig Uterus.—With the virgin uterus, S 1 in 1,000 invariably causes an immediate tonic contraction, sustained for at least two minutes. The degree of contraction, shape of the contraction curve, and duration of effect depend not only upon the concentration of extract but also upon the phase of the oestrous cycle. Uterus from a guinea-pig in oestrus is more sensitive than uterus removed at other periods of the cycle. Consequently we have found it advantageous to keep a number of spayed animals and to induce oestrus, as required, by inoculations of stilboestrol. With such sensitized preparations concentrations of 1 in 2,000 or even 1 in 10,000 S may produce contraction; concentrations of 1 in 500 or 1 in 200 cause maximal contraction, sustained for more than 20 minutes and without the stepladder effect produced by smaller doses. Washing out the bath is always followed by a return of the normal tone and rhythmical contractions of the muscle. The uteri of pregnant guinea-pigs respond in the same way as those from virgin animals, but may be either more or less sensitive. The differences probably depend upon the period of gestation and may be related to hormonal balance, but the data so far available are not sufficient to correlate these factors. Experiments with parturient uteri give results in striking contrast: the extract produces

FIG. 1.—Effect of S on guinea-pig uterus. A, Oestrus induced; sustained contraction. B, Parturient; contraction followed by relaxation.

only a small contraction of short duration, which is followed by prolonged abolition of uterine tone. No fewer than 64 guinea-pig tests have been made, with perfectly consistent results except for one or two cases in which the uteri were of such low sensitivity that effects were negligible. The effect of the extract on guinea-pig uterus is not influenced by previous addition of atropine to the bath, nor does the extract modify the response obtained with adrenaline. Typical experiments are illustrated in Fig. 1.