EFFECT OF VALYL-OXYTOCIN ON THE HUMAN SUBJECT

BY

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Following their recent methods for the synthesis of oxytocin ("syntocinon"), Boissonnas *et al.* (1956) published an account of their synthesis and the biological properties of some analogues of oxytocin in which one amino-acid residue had been replaced by another. In a subsequent publication Berde *et al.* (1957) dealt in detail with the pharmacological properties of four of these compounds. They were interesting because when assayed against the international pituitary (posterior lobe) standard powder their relative activity varied according to the method of assay chosen.

In three of the analogues the isoleucyl group of oxytocin was replaced by a phenylalanyl, leucyl, or valyl residue, the compounds being designated P-, L-, or V-analogue respectively. In the fourth, named the G-analogue, the asparaginyl group was replaced by a glutamyl residue. The results of the biological asssay of synthetic oxytocin and its four synthetic analogues are summarized in the accompanying Table.

The differences in the results were surprising, especially as synthetic oxytocin, made by the method of Boissonnas *et al.* (1955), and the natural mammalian pituitary preparation ("pitocin") had given identical assays in all species tested and in *in-vitro* experiments, and had also proved indistinguishable in the human (Bainbridge *et al.*, 1956).

It therefore seemed desirable to compare one of the above-mentioned analogues with oxytocin in its effects on the human subject, and thereby to see which of the biological assays was the better representative of potency in the human. It seemed possible that these results might confirm or disprove the suitability of present pharmacopoeial methods for the assay of oxytocin or its analogues.

Valyl-oxytocin was chosen for the human tests—that is, the V-analogue—and the findings form the subject of this article.

Comparisons between synthetic oxytocin (syntocinon) and valyl-oxytocin have been made as described below; one unit of either drug being a unit according to the *B.P.* method of assay against the international standard pituitary powder.

Intrauterine-pressure Changes

Therapeutic Abortion. — Intrauterine-pressure changes were recorded in a case of therapeutic abortion at 19 weeks' gestation. No anaesthetic was given, and the injections were made intravenously. A three-point matching assay showed 0.25 unit of syntocinon to be slightly stronger than 0.05 unit of valyl-oxytocin (Fig. 1).

Patients With Dead Foetus. — Intrauterine-pressure changes were recorded on two patients with a dead foetus at approximately 28 weeks' gestation. By giving intramuscular injections at two-hourly intervals it was shown that, for the same initial rise of tone and to maintain rhythmical contractions for about two hours, the first patient required three units of syntocinon or 0.8 unit of valyl-oxytocin. In the second patient 2 units of syntocinon produced a greater rise of tone than 0.25 unit of valyl-oxytocin and less than that produced by 0.75 unit of valyl-oxytocin. The contractile rhythms produced by valyl-oxytocin persisted longer and were more regular than those produced by syntocinon, an observation also to be seen in Fig. 1. The results for the second patient are reproduced in Fig. 2.

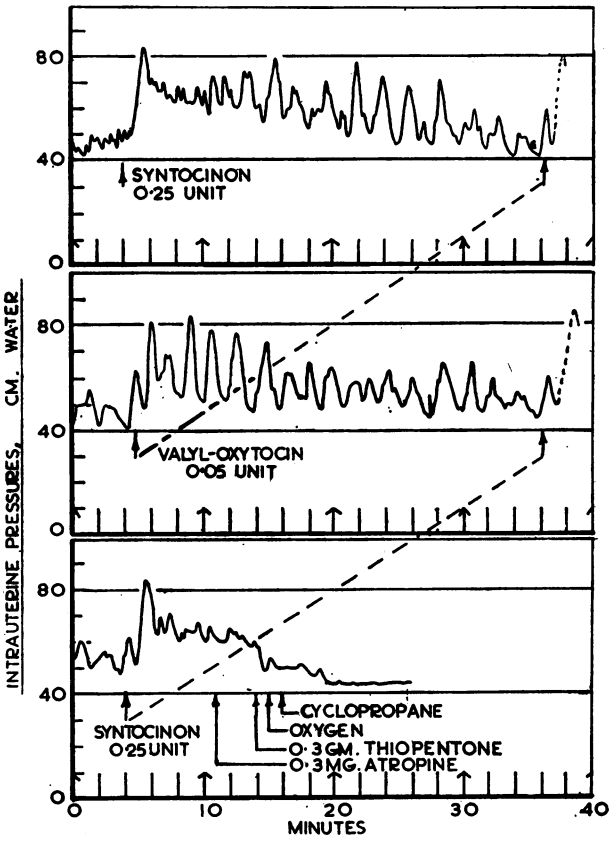

FIG. 1.—Three-point matching assay of intravenous syntocinon and valyl-oxytocin in an unanaesthetized patient sedated with "omnopon" 10 mg., scopolamine 0.3 mg., and chlorpromazine 12.5 mg.; intramuscular injection 30 minutes before start of assay. Prior to therapeutic abortion at 19 weeks' gestation. Pressures from 2-ml uterine balloon.

Activity of 1 ml. of Solutions of the Cyclic Octapeptides in Units of International Standard Pituitary (Posterior Lobe) Powder in Different Tests (from Berde et al., 1957)

	Isolated Rat Uterus	Chicken Blood Pressure	Milk-ejection Pressure (Rabbit Mammary Gland)	Cat Uterus *in situ*	Antidiuretic Activity (Non-anaesthyetized Rats)	Pressor Activity (Spinal Cats)
Synthetic oxytocin	8·0 (±0·22)	8·1 (±0·16)	8·3 (±0·23)	10·7 (±0·9)	0·09 (±0·05)	0·07
P-analogue	2·7 (±0·11)	2·2 (±0·08)	5·8 (±0·37)	2·4 (±0·3)	2·8 (±1·2)	0·5
L-analogue	0·25 (±0·01)	0·33 (±0·01)	2·2 (±0·44)	2·3 (±0·4)	0·15 (±0·08)	0·15
V-analogue (valyl-oxytocin)	2·8 (±0·11)	3·2 (±0·08)	15·0 (±0·75)	16·7 (±1·3)	0·04 (±0·02)	0·015
G-analogue	<0·0025	<0·02	0·025 (±0·003)	0 (Up to 1 ml./kg. i.v.)	0 (Up to 1 ml./100 g. s.c.)	0 (Up to 0·5 ml./kg. i.v.)

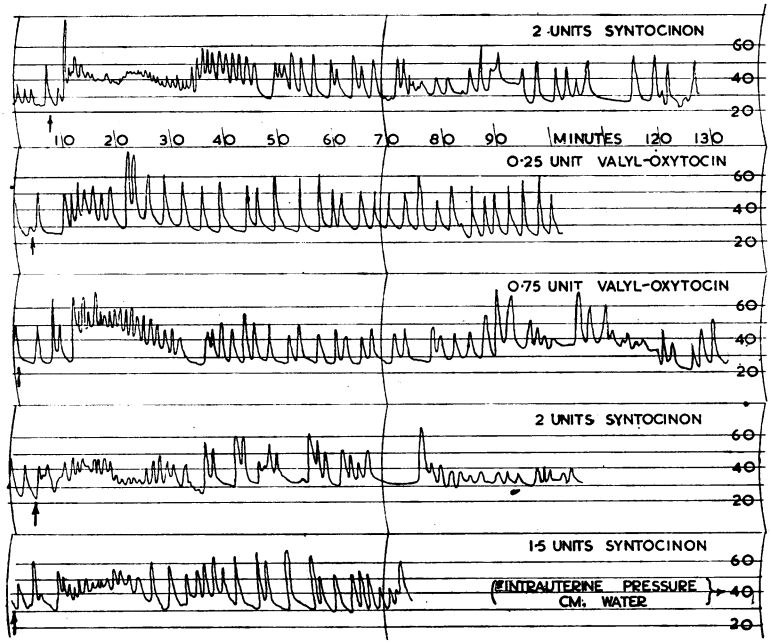

FIG. 2.—Intrauterine pressure produced by intramuscular injections of syntocinon and valyl-oxytocin compared. 28 weeks' gestation; dead foetus.

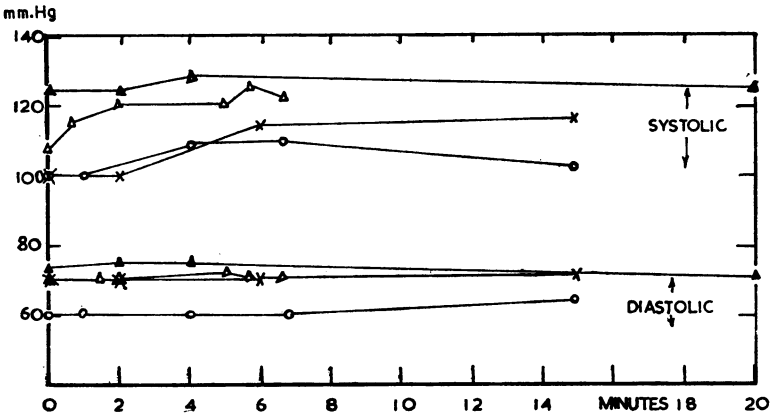

FIG. 3.—Effect of intravenous valyl-oxytocin on blood pressure in four post-partum patients. Doses: O and X, 0.5 unit; △ and ▲, 0.25 unit.

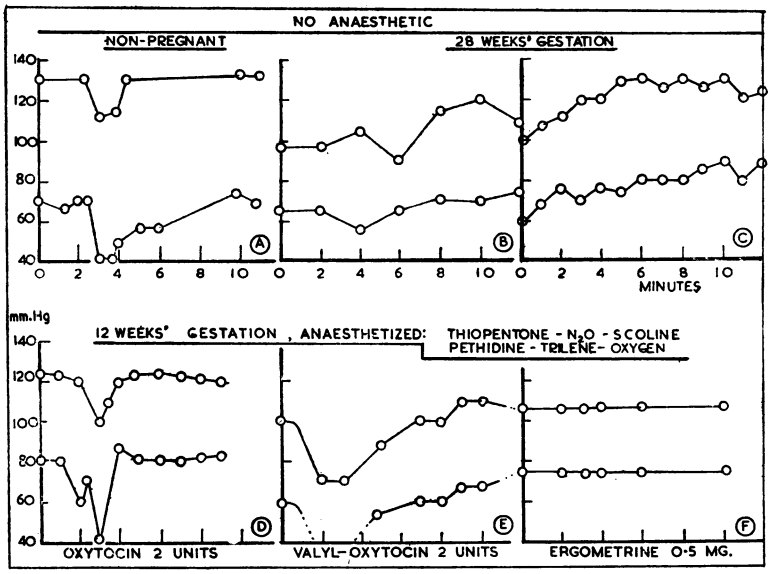

FIG. 4.—Oxytocics, intravenous injections, and blood pressure. Curves A-F are explained in the text.

Blood-pressure Changes

The blood pressure was recorded during the courses of the intramuscular injections given in Section 2, above. Large intramuscular doses of syntocinon, 2 or 3 units, caused a gradual diminution in blood pressure from values of 100/70 to 70/60 mm. Hg, whereas equiactive oxytocic doses of valyl-oxytocin, 0.5 to 0.8 unit, resulted in a slight rise in blood pressure to 115/75 mm. Hg. These findings led to the making of more exact comparisons with intravenous doses, the results of which are reproduced in Figs. 3 and 4.

Fig. 3 shows the result of giving 0.25 and 0.5 unit of valyl-oxytocin intravenously to four patients on the third post-partum day. The blood pressure was taken every minute for five minutes, then every five minutes for 15 minutes. No fall in pressure was demonstrated, and in three patients there was a rise in systolic pressure. These injections induced uncomfortable uterine contractions lasting throughout the test, and the associated uterine ischaemia may well account for the slight pressure rise. Fig. 4 demonstrates that a sudden drop in blood pressure can be provoked by larger doses of valyl-oxytocin. In this respect valyl-oxytocin resembles oxytocin, and the test appears to parallel the pharmacopoeial assays, since about 2 units of either drug is required to produce this effect.

Curve A of Fig. 4 is reproduced from Mayes and Shearman (1956) and curve D from Schild *et al.* (1951). The other four curves were made at the present time. Curves B and C were on the same patient on successive days, while curves E and F were made sequentially on another patient prior to evacuation of the uterus. Where the pregnancy is advanced and there is a large uterine blood supply the fall in blood pressure tends to be masked by the rise brought about by the simultaneous uterine contraction, but a distinct kink is noticeable in curve B. These curves are particular examples, and doubtless many differences would be found between these and patients

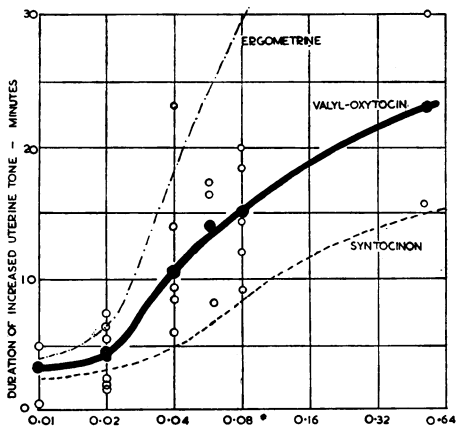

FIG. 5.—Uterine tone produced by intravenous injections of valyl-oxytocin in post-partum patients compared with those of syntocinon and ergometrine. ●=Averages. O=Observations with valyl-oxytocin. Dose: Syntocinon and valyl-oxytocin in units; ergometrine in mg.

with abnormally raised blood pressure, toxaemia of pregnancy, and in different stages of gestation or the puerperium.

Changes in Uterine Activity

Valyl-oxytocin was given to 30 patients on the second and the third post-partum day with simultaneous recording of uterine activity by means of an abdominal tocodynamometer. The drug was given intravenously after a baseline of uterine activity had been recorded for about 20 minutes. The change in uterine activity recorded in this way with oxytocin injections shows a response which is very variable in height, a factor dependent on the uterine contents and whether the uterus hardens round the blood clot within or expels it. The duration of raised uterine tone, however, shows a graded relation to dose, and this has been plotted in Fig. 5. The method is the same as that used to assay syntocinon (Bainbridge *et al.*, 1956). In Fig. 5 the curve for syntocinon is also reproduced, and a curve has been constructed from an ergometrine assay (Myerscough and Schild, 1955). Valyl-oxytocin is seen to occupy an intermediate position between these two curves. Depending on the dose, valyl-oxytocin is between two and five times as long-acting as oxytocin in raising uterine tone. Three typical records are reproduced in Fig. 6.

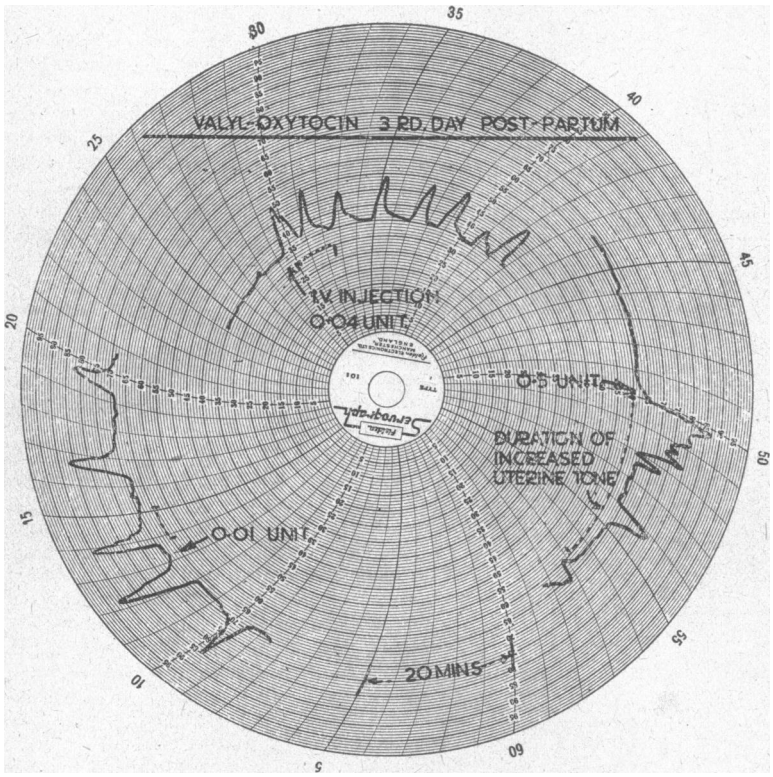

FIG. 6.—Three typical records of effect of valyl-oxytocin in raising uterine tone.

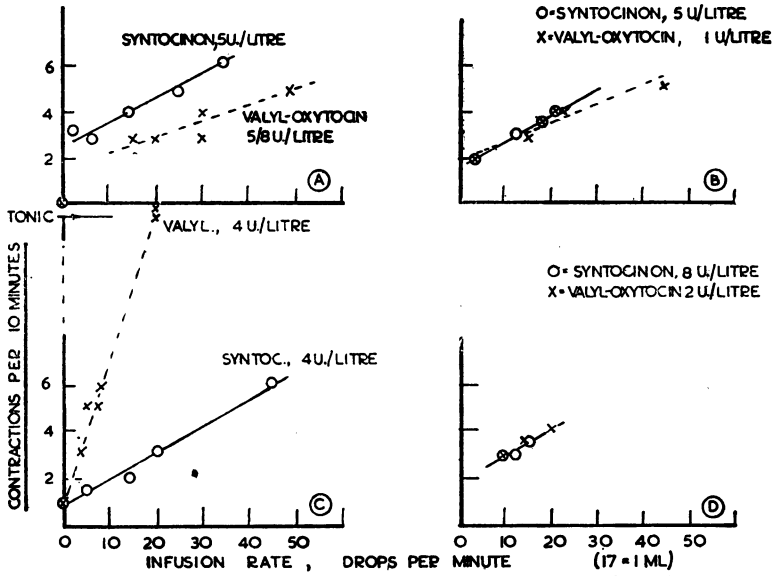

FIG. 7.—Records of intravenous injections of valyl-oxytocin, in 5% dextrose, in four intra-partum patients at term. Curves A–D are explained in the text.

Effect on Parturient Women

Valyl-oxytocin was given to eight parturient patients by intravenous infusion employing concentrations varying from 0.5 to 4 units per litre of 5% dextrose. It behaves similarly to oxytocin, readily producing a good contractile rhythm if the infusion rate is suitably adjusted. No abnormal effects were noticed, and the babies did not show any signs that could be attributed to intrauterine anoxia or abnormal pressures. A matching assay was carried through on four of these patients who were given synthetic oxytocin and valyl-oxytocin infusions alternately, and the infusion rates were adjusted to provide various rates of contraction; at least four changes were made on each patient. The first result of the infusion is to start a contractile rhythm, if one is not already present. Increasing the infusion rate increases the rate of contractions, and, eventually, further increases in rate cause the tone between contractions to rise. A rise in tone is not considered desirable, since it may lead to foetal anoxia, and the assay is not intentionally pursued to such concentrations.

The results of the four assays are reproduced in Fig. 7. In equal concentrations valyl-oxytocin was considerably more potent than oxytocin (curve C). Diluted eight times (curve A), valyl-oxytocin was noticeably less potent. Diluted 5 times (curve B, valyl-oxytocin was slightly less potent, and at four-times dilution there was no perceptible difference (curve D). Not only do these four assays point to the same conclusion, but they are consistent numerically between themselves to an accuracy of + or − 10%.

Discussion

The assay shows that, as an excitor of contractions, valyl-oxytocin is four times as potent as synthetic oxytocin in the human subject even though the two substances are equiactive when assayed by the pharmacopoeial method—that is, by depression of the blood pressure in a chicken and the rat uterus method.

If, however, valyl-oxytocin is diluted four times so as to be equiactive with oxytocin in the human subject, then it is also practically equiactive in terms of the rise in milk ejection pressure in the rabbit mammary gland and in its action on the cat uterus *in situ*. Synthetic and natural oxytocin give roughly identical results when tested by any of the above methods, and therefore it appears that the milk-pressure assay, or that of cat uterus *in situ*, might with advantage be used in addition to the pharmacopoeial methods.

Doses of valyl-oxytocin which are equiactive in the human subject with oxytocin

in exciting uterine contractions are not equiactive in their effects on blood pressure. Blood pressure is affected only by extremely large doses of oxytocin, 2 units intravenously usually being required to produce a pressure fall lasting about a minute and occurring two to three minutes from the time of injection. Half a unit of valyl-oxytocin does not produce this effect, but 2 units will do so. In this respect the *B.P.* assay of the two substances on chicken blood pressure or isolated rat uterus is parallel to the comparison in the human subject so far as the limited tests we have made are able to show.

Summary

Following their synthesis of oxytocin, Boissonnas *et al.* (1956) prepared analogues of oxytocin having actions which were similar qualitatively but which differed quantitatively.

A comparison has been made of the actions of one of these analogues, valyl-oxytocin, with those of synthetic oxytocin in animals and in man.

The results are discussed, with special reference to the present methods of assay.

The oxytocin and valyl-oxytocin used in this work were generously supplied by Messrs. Sandoz, Basle, Switzerland. Dr. H. Holgate, of Sandoz, London, has kindly supplied us with the published information on the analogues of oxytocin. The preparations of oxytocin and valyl-oxytocin have been standardized by the Sandoz Laboratories, Basle, against the International Standard Pituitary Powder. Our thanks are due to the editor and publishers of the *British Journal of Pharmacology and Chemotherapy* for permission to reproduce the Table, and to the editor of the *Lancet* and of the *Journal of Obstetrics and Gynaecology of the British Empire* for permission to reproduce Figs. 4a and 4d.

In the post-partum assays I was grateful for the assistance of Miss M. N. Bainbridge and also of Miss J. Farrow, who assisted with the intravenous infusion assays. The staff of the Obstetric Unit, and in particular the Director, Professor W. C. W. Nixon, encouraged and assisted the work in many respects. Dr. H. O. Schild, reader in pharmacology, University College, London, has given generous assistance in planning the work.

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"Recent work on the hormonal prevention of conception in man by Pincus and his colleagues has depended on the suppression of ovulation and has been made practicable by the preparation of orally-active substances with progesterone-like activity. One of these seems to be promising in Pincus's clinical experiments. Presumably it overwhelms the follicular phase of the cycle partly by local inhibition and partly by interference with pituitary activity. It seems, however, that treatment is necessary over some 20 days in each cycle, probably in order to ensure that ovulation is prevented and not merely postponed, and for this and other reasons this work cannot yet be said to have put oral contraception on a practical basis. But it is most promising and exciting." This is from an article by Dr. A. S. PARKES, F.R.S., "Towards Oral Contraception," which describes the origin and work of the Oliver Bird Trust and the Council for the Investigation of Fertility Control (*Family Planning*, 1958, 7, 9). *Family Planning* is published quarterly and is obtainable by non-members from the Family Planning Association, 64, Sloane Street, London, S.W.1, price 2s. 6d. (post free) annually.

PROLONGED REMISSION IN CHRONIC LYMPHATIC LEUKAEMIA

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The duration of life of patients with chronic lymphatic leukaemia has been variously quoted, but Tivey (1954), in an analysis of 46 series of cases reported in the literature between 1925 and 1951, estimated the median survival to be about 1.6 years after commencement of therapy and 2.6 years after the onset of the first clinical symptoms. He found, however, that the survival curves were "badly skewed," and it is generally agreed that individual patients can live for a very much longer time. Amongst the longest survivors with chronic lymphatic leukaemia recorded is one of 29 years (Marlow and Bartlett, 1953) and one of 25 years (McGavran, 1938).

A number of cures of leukaemia have been reported in the literature. According to Forkner (1938) and Wintrobe (1956), in only a few can the possibility of a cure be considered. Schott (1955) describes the case of a patient with chronic lymphatic leukaemia who was symptom-free and had a normal peripheral blood count 10 years after radiation treatment.

The following case is presented as a further example of long-term remission in a patient suffering from chronic lymphatic leukaemia who is clinically well and has a normal peripheral blood count eight years after the last treatment. Marrow punctures have been carried out at various intervals during the time of observation, and are discussed below. An interesting additional feature is the development in this patient of carcinoma of the cervix, treated apparently successfully three years ago.

Case History

A married woman, then aged 49, was admitted to another hospital on February 23, 1946, with 2 weeks' history of weakness and giddiness, and 1 week's continuous vaginal bleeding following six months' amenorrhoea. There was no previous significant illness. She had had nine normal confinements. On examination at that time pallor was marked, there was a moderate generalized lymphadenopathy, and the liver and spleen were palpable. Pelvic examination did not reveal any local cause for her vaginal bleeding.

Investigations.—R.B.C., 770,000/c.mm.; haemoglobin, 2.5 g./100 ml.; W.B.C., 27,000/c.mm. (polymorphs 4%, eosinophils 1%, lymphocytes 93%, monocytes 2%). There were no nucleated red cells. Platelets, 31,000/c.mm. Bleeding-time, 10 minutes. Clotting-time, 8 minutes.

Puncture of the sternal marrow confirmed the diagnosis of lymphatic leukaemia. She was given repeated transfusions, and from March 4 to 28 was treated with urethane. This was followed by a profound fall in the W.B.C. and a tendency to spontaneous bleeding from the gums, with infection of the mouth and throat. Further transfusions, penicillin, "anahaemin" injections, and ferrous sulphate were given, with slow recovery (Fig. 1). She was discharged from hospital on August 3. Her attendance thereafter for follow-up was erratic.