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SOME PHARMACOLOGICAL ACTIONS OF FOUR SYNTHETIC ANALOGUES OF OXYTOCIN

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Four structural analogues of oxytocin were investigated with regard to their oxytocic, milk-ejecting, pressor and diuretic/antidiuretic effects. In three of them the isoleucyl group of oxytocin was replaced by a phenylalanyl, leucyl, or valyl residue; in the fourth the asparaginyl group was replaced by a glutaminyl residue. Synthetic oxytocin and the international standard pituitary (posterior lobe) powder were used for comparison. Although the analogues showed marked differences in their oxytocic effects, there was a fairly good agreement between the results obtained on the isolated rat uterus and the blood pressure of the chicken for each polypeptide. The milk-ejection pressure test gave much higher values throughout. The pressor and antidiuretic activities of the four analogues showed no obvious correlation with the values obtained in the other tests. The valyl and the leucyl analogues also had a diuretic effect. The *phenylalanyl* analogue was remarkable for the close correspondence between its oxytocic and antidiuretic effects: practically identical values were obtained for the potency, whether measured on the rat uterus *in vitro*, the blood pressure in the chicken, the cat uterus *in situ* or water diuresis in the rat. The *leucyl* analogue showed an oxytocic activity on the cat uterus *in situ* or the rabbit mammary gland roughly 7 to 9 times as high as that measured by means of the conventional bioassay methods, such as the blood pressure of the chicken or the rat uterus *in vitro*. The *glutaminyl* analogue, the weakest of the whole series, had only a modest effect on the mammary gland. The *valyl* analogue was the most interesting of the new polypeptides. Its oxytocic action on the cat uterus *in situ* and its milk-ejecting effect were greater than that of synthetic oxytocin, whereas its antidiuretic and pressor effects were less. In cats and rats, the uterine effect was stronger *in situ* than *in vitro*. There were also distinct species differences between cats, rabbits, and rats in their sensitivity to valyl-oxytocin.

Methods recently developed for the synthesis of oxytocin (du Vigneaud, Ressler, Swan, Roberts, Katsoyannis, and Gordon, 1953; Boissonnas, Guttmann, Jaquenoud, and Waller, 1955) have not only made available preparations of this hormone containing no vasopressin, but also permitted the synthesis of structural analogues of oxytocin. A series of such cyclic octapeptides (Table I) has been prepared by Boissonnas, Guttmann, Jaquenoud, and Waller (1956a), the isoleucyl group of oxytocin being replaced by a phenylalanyl, leucyl or valyl group. The valyl derivative (" valyl-oxytocin ") proved to be the most active of these three analogues of oxytocin. Some of its properties have already been briefly described (Boissonnas, Guttmann, Jaquenoud, Waller, Konzett, and Berde, 1956b). A fourth analogue of oxytocin has been obtained by replacing the asparaginyl group by a glutaminyl group (Boissonnas, Guttmann, Jaquenoud, and Waller, 1956a).

In the meantime, Rudinger, Honzl, and Zaoral (1956), in a preliminary communication, reported the synthesis of two analogues of oxytocin having a leucyl or valyl group instead of the isoleucyl group; and du Vigneaud (personal communication) prepared an analogue of oxytocin having a phenylalanyl group instead of the isoleucyl group (" oxypressin ").

The investigations described below are concerned with the effects of the four analogues of oxytocin prepared by Boissonnas, Guttmann, Jaquenoud, and Waller (1956a). For simplicity, these will be referred to as the phenylalanyl (P), leucyl (L), valyl (V) and glutaminyl (G) analogues of oxytocin (Table I). A synthetic preparation of oxytocin (Syntocinon, Sandoz), described by Boissonnas, Guttmann, Jaquenoud, and Waller

TABLE I

THE SEQUENCE OF THE AMINO ACIDS IN OXYTOCIN AND ITS ANALOGUES

Synthetic oxytocin	CyS—Tyr—Ileu—Glu(NH_2)—Asp(NH_2)—CyS—Pro—Leu—Gly(NH_2)
P-analogue	CyS—Tyr—*Phe*—Glu(NH_2)—Asp(NH_2)—CyS—Pro—Leu—Gly(NH_2)
L-analogue	CyS—Tyr—*Leu*—Glu(NH_2)—Asp(NH_2)—CyS—Pro—Leu—Gly(NH_2)
V-analogue (" valyl-oxytocin ") ..	CyS—Tyr—*Val*—Glu(NH_2)—Asp(NH_2)—CyS—Pro—Leu—Gly(NH_2)
G-analogue	CyS—Tyr—Ileu—Glu(NH_2)—*Glu*(NH_2)—CyS—Pro—Leu—Gly(NH_2)

(1955) and Konzett, Berde, and Cerletti (1956), and the international standard pituitary (posterior lobe) powder were used for comparison.

These polypeptides were first tested on the isolated rat uterus and on blood pressure in the chicken, both being methods recommended in pharmacopoeias for oxytocin assay, and on the milk-ejection pressure of the rabbit mammary gland. All three assay methods yielded quantitative data. However, it became evident that further tests were necessary to distinguish between these compounds. The study was therefore extended to include investigations on the cat uterus *in situ* and *in vitro*, on the rat uterus *in situ*, on the induction of labour in non-anaesthetized pregnant rabbits, on the blood pressure of spinal cats and on urine secretion in non-anaesthetized rats. Quantitative results with well-defined limits of error were provided by many of these methods, for example the cat uterus *in situ* and *in vitro*, the induction of labour in non-anaesthetized rabbits and the antidiuresis in non-anaesthetized rats. The other methods, such as the rat uterus *in situ*, the blood pressure in spinal cats and the diuresis in non-anaesthetized rats, provided a rough measure of the activity, and more information on the type of action, of these polypeptides.

METHODS

Solutions.—To obtain the cyclic octapeptides, 20 mg. of the corresponding nonapeptide (Boissonnas, Guttmann, Jaquenoud, and Waller, 1956a) was dissolved in 5 ml. liquid ammonia and reduced with sodium (du Vigneaud, Ressler, Swan, Roberts, and Katsoyannis, 1954). Ammonium chloride (5 mg.) was added; the ammonia was evaporated and the residue added to 100 ml. of water, the *p*H adjusted to 6.8, CO_2-free air bubbled through the solution for 2 hr., and the *p*H adjusted to 4.5. Saline was used for further dilution.

Isolated Rat Uterus.—On the day preceding the experiment the rats were treated with 0.2 mg. stilboestrol subcutaneously to increase the sensitivity of the uterus. The assays and the statistical treatment of the results were carried out according to the method of Holton (1948).

Chicken Blood Pressure.—The method of Coon (1939), using white Leghorn roosters, was followed; for the statistical analysis the procedure of Thompson (1944) was utilized.

Milk-ejection Pressure of the Rabbit Mammary Gland.—The pressure within the lactating mammary gland of rabbits was measured by the method of van Dyke, Adamsons and Engel (1955), modified according to Berde and Cerletti (1956b). The tests were made between the 3rd and 27th day *post partum*. The anaesthetics used were urethane (1.25 g./kg., subcutaneously) with pentobarbitone (10 mg., intravenously) given as a supplement to urethane whenever necessary. Artificial respiration was provided in many experiments but not in all. One of the ducts in a nipple was cannulated and connected to a Statham strain-gauge manometer, the milk-ejection pressure being recorded on a photokymograph. After preliminary trials, the final test was carried out as a four-point assay (Schild, 1942), the doses of the standard and of the unknown substance being injected intravenously at intervals of 3 min.

Rat Uterus in situ.—Rats treated with stilboestrol (0.2 mg., subcutaneously) on each of 2 days preceding the experiment were anaesthetized with urethane (1.4 g./kg., subcutaneously). A midline incision was made and one horn of the uterus was attached to a string by means of a suture through the connective tissue near the end of the horn. The string was led over a pulley and attached to the short arm of a frontal writing lever which recorded the contractions of the uterus on a smoked drum (magnification × 20). To prevent the horn of the uterus from drying, it was covered by loosely suturing the skin which had been divided by the midline incision. Injections were made into the jugular vein at intervals of 60 min. The reactions to identical doses of the international pituitary standard (0.05 to 0.2 *I.U.*) often varied greatly in the same experiment, but in good preparations the response remained fairly constant. A double dose elicited a much stronger contraction. Various doses

of the unknown preparation and a constant dose of the standard were given alternately. The effectiveness of the unknown preparation was estimated by "bracketing" doses of the unknown with doses of the standard. The results obtained did not permit an accurate determination of the limits of error.

Cat Uterus in situ.—Non-pregnant animals were anaesthetized with a mixture of urethane (0.428 g./kg.) and chloralose (0.0428 g./kg., subcutaneously). Most of them received no pretreatment; a few were given α-oestradiol and progesterone, as recommended by Clary, Cameron, and Craver (1951). The uterus was exposed by a median incision. A thread was looped round one of the uterine horns and attached to a lever fitted with a frontal writing lever (magnification ×3). Here again the tests were based on the technique of "bracketing" constant doses of the standard with differing doses of the unknown preparation. The activity of the unknown substance and the approximate standard error were estimated by the method of Miller and Tainter (1944).

Cat Uterus in vitro.—Uterine segments (1 to 2 cm. in length) of non-pregnant animals were suspended in a 50 ml. bath containing oxygenated Tyrode solution with 0.1% glucose, and attached to a lever weighing 1 to 5 g. (magnification ×3). Otherwise these tests were carried out in the same way as those on the cat uterus *in situ*.

Induction of Labour in the Rabbit.—It is known that pregnant rabbits near to term frequently go into labour if posterior pituitary extract (Knaus, 1926) or oxytocin (Csapo, 1955) is injected. Non-anaesthetized rabbits were used on the morning of the 31st day of pregnancy (exactly 720 hr. after mating). The substances to be tested were injected intramuscularly. The induction of labour was considered successful if the first foetus was delivered within 60 min. of the injection; as a rule, this happened within a few minutes if the dose administered was effective. By contrast, only 1 of 25 control animals injected with saline solution delivered within 1 hr. Three doses of each substance were tested, each dose being given to a group of 20 or 25 rabbits. The logarithm of the dose was plotted against the probit effect in order to determine the ED50 and its approximate standard error, as described by Miller and Tainter (1944).

In addition to these estimations of oxytocic activity, further investigations were performed to estimate the effect of the polypeptides on blood pressure and urine secretion.

Pressor Activity.—Spinal cats were used. Here again the technique of "bracketing" doses of the unknown substance with doses of the standard was used. The results obtained did not permit accurate determination of the limits of error.

Effect on Urine Secretion.—A slightly modified form of the water diuresis test of Burn (1931, 1950) was carried out on non-anaesthetized male rats weighing 130 to 200 g. On the evening preceding the experiment all food was removed from the cage. The following morning the animals received 2.5 ml./100 g. tap-water at body temperature by stomach tube. A loading dose of 5 ml./100 g. of water was given by stomach tube 2½ hr. later, when the test preparation was injected subcutaneously. In all other respects the experiment and the calculation of the time of maximum rate of excretion were carried out according to Burn's recommendations. The results gave a measure of the antidiuretic effect. Once the approximate activity of a substance had been established the final test was carried out as a four-point assay (Schild, 1942).

The relation between the loading amount of water and the total quantity of urine excreted during the period of enhanced diuresis was calculated in order to detect a possible diuretic effect (Fraser, 1937).

RESULTS

Table II summarizes most of the results obtained with synthetic oxytocin and its four synthetic analogues. Identical values were obtained for the activity of synthetic oxytocin when it was assayed on the isolated rat uterus, on blood pressure in the chicken or on the milk-ejection pressure of the rabbit mammary gland. When tested on the cat uterus *in situ* an activity about 30% higher than that obtained with other methods was found. A certain amount of antidiuretic and pressor activity was inherent in synthetic oxytocin, but it was only equivalent to about 1% of the oxytocic potency.

TABLE II

ACTIVITY OF 1 ML. OF SOLUTIONS OF THE CYCLIC OCTAPEPTIDES IN UNITS OF INTERNATIONAL STANDARD PITUITARY (POSTERIOR LOBE) POWDER IN DIFFERENT TESTS

	Isolated Rat Uterus	Chicken Blood Pressure	Milk-ejection Pressure (Rabbit Mammary Gland)	Cat Uterus *in situ*	Antidiuretic Activity (Non-anaesthetized 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.)

There was a fairly good correlation between the effect of each analogue on the isolated rat uterus and the chicken blood pressure, but the effects in the milk-ejection pressure test were greater. This was true even of the G-analogue, which had practically no detectable effect on the isolated rat uterus and chicken blood pressure but showed some effect on milk-ejection pressure. The effect of the four compounds on the cat uterus *in situ* followed a different pattern. Whereas the L- and V-analogues exhibited comparable effects on the milk-ejection pressure and on the cat uterus *in situ* (with a tendency for the effect on the latter to be slightly increased) the P- and G-analogues were less active on the cat uterus *in situ* than on the milk-ejection pressure.

The pressor and antidiuretic activities of the four analogues bore no obvious relation to the values obtained in the other tests. The P-analogue had a marked pressor activity and a remarkably great antidiuretic effect whereas the other compounds are much less active. If the total quantity of urine excreted in Burn's test during the period of enhanced diuresis was taken into consideration, a characteristic pattern of reaction to three of the synthetic polypeptides was revealed. The rats treated with 0.016–0.5 ml./100 g. synthetic oxytocin, 0.1 and 0.2 ml./100 g. valyl-oxytocin or 0.03 and 0.06 ml./100 g. of the L-analogue excreted significantly more urine than those treated with various doses of the international standard powder or the controls which received 0.2 ml./100 g. saline. No such diuretic reaction was seen after the administration of the P- and G-analogues.

The most interesting of the new polypeptides was valyl-oxytocin. It had an effect greater than that of synthetic oxytocin on milk-ejection pressure and on the cat uterus *in situ*. The antidiuretic and the pressor effects, however, were weaker than those of synthetic oxytocin. Further investigation of this compound on the cat uterus *in vitro* and on the rat uterus *in situ* revealed that, in both species its effect on the uterus *in situ* was much greater than on the uterus *in vitro*. The potency of valyl-oxytocin on the isolated rat uterus was 2.8 ($\pm$0.11) *I.U.*/ml., and on the rat uterus *in situ* about 10 *I.U.*/ml. The values for the isolated cat uterus were 6 ($\pm$0.6) *I.U.*/ml. and for the cat uterus *in situ* 16.7 ($\pm$1.3) *I.U.*/ml. Although the results obtained in the studies on the rat uterus *in situ* did not permit a statistical analysis to be made, the difference between the oxytocic action of valyl-oxytocin *in vitro* and *in situ* existed in both species.

The activity of valyl-oxytocin in inducing labour in rabbits near term was found to be equivalent to 6.2 ($\pm$3) *I.U.*/ml. and that of synthetic oxytocin to be 9.3 ($\pm$4.1) *I.U.*/ml.

Discussion

The present investigations on some pharmacological actions of synthetic oxytocin and four synthetic oxytocin analogues show that the substitution of one amino acid by another in the oxytocin molecule resulted in cyclic polypeptides with somewhat different effects. This is not surprising in view of the fact that replacement of two amino acids in the oxytocin molecule by two others (replacing isoleucine by phenylalanine, and leucine by arginine or lysine) yields vasopressin (du Vigneaud, Lawler, and Popenoe, 1953).

The P-analogue, in which the phenylalanyl group is in the same position as in the bovine and the porcine types of vasopressin, and which therefore has a cyclic part identical with that of vasopressin, exhibits a weaker oxytocic effect than oxytocin and enhanced antidiuretic and pressor effects. The antidiuretic and pressor activity of the P-analogue is greater than that of the other preparations in the series. There is, however, a discrepancy between the potency as measured by the antidiuretic effect and that measured by the pressor effect of the P-analogue. This seems to be an indication of the relative importance of arginine and lysine for the pressor effect of vasopressin. In the milk-ejection pressure test, the P-analogue showed a remarkable potency of more than twice that measured on the isolated rat uterus, the chicken blood pressure and the cat uterus *in situ*.

Du Vigneaud's highly purified P-analogue (" oxypressin ") also showed its maximum activity in the milk-ejection pressure test (du Vigneaud, personal communication). As the details of the biological investigation with du Vigneaud's oxypressin are not yet available, a quantitative comparison between the potencies of oxypressin and our P-analogue in different tests would be premature.

With regard to the L-analogue, it is surprising to find that the oxytocic effect on the cat uterus *in situ* is about 9 times as great as that on the rat uterus *in vitro*. The pronounced effect on the cat uterus *in situ* is in accord with the effect on milk-ejection pressure. The antidiuretic activity of the L-analogue, however, is remarkable. The ratio between antidiuretic effect and oxytocic effect (on the isolated rat uterus or on the cat uterus *in vivo*)

is definitely greater for the L-analogue than for synthetic oxytocin.

The G-analogue exhibits such a low activity that definite values could only be obtained in the milk-ejection pressure test. The substitution of glutamic acid for aspartic acid results in far greater changes in activity than is obtained by replacing the iso-leucyl group by other amino acids.

The V-analogue, which we (Boissonnas, Guttmann, Jaquenoud, Waller, Konzett, and Berde, 1956b) suggested be termed " valyl-oxytocin," is of great interest because of its high oxytocic potency *in vivo* and its low vasopressor and antidiuretic activity. If the values obtained in studies on the cat and rat uterus *in situ*, the induction of labour in rabbits and the milk-ejection pressure test in the rabbit are compared with those obtained for water diuresis in non-anaesthetized rats and blood pressure in spinal cats, the ratio of the oxytocic to the pressor-antidiuretic potency is greater for valyl-oxytocin than for oxytocin.

In cats and rats there is a marked discrepancy between the effect of valyl-oxytocin on the uterus *in situ* and *in vitro*. It is not yet possible to give an explanation for this phenomenon, especially as synthetic oxytocin and the P-analogue do not exhibit the same difference in activity on the uterus *in vitro* and *in situ*.

Rudinger, Honzl, and Zaoral (1956) in their preliminary communication state that their L- and V-analogues of oxytocin " showed uterus-contracting, avian vasodepressor and milk-ejecting activities lower by about one order of magnitude than the corresponding activities of oxytocin." They do not mention the marked effect of the valyl analogue of oxytocin on milk-ejection pressure. The same authors say that " the antidiuretic effect of the valine analogue was about equal to that of the natural hormone, the activity of the leucine derivative some ten times greater." Since the methods used in these studies are not given in detail, no comment can be made on the discrepancy between their findings and ours.

It is remarkable that synthetic oxytocin and its analogues exhibit a very high degree of activity in the milk-ejection pressure test. This method has already been described as highly sensitive for oxytocin (van Dyke, Adamsons, and Engel, 1955). It seems that the myoepithelial cells of the rabbit mammary gland, which bring about the increase in the milk-ejection pressure (Linzell, 1955), are also highly sensitive to all the synthetic analogues of oxytocin included in this study.

The diuretic action of synthetic oxytocin and two of its analogues (valyl-oxytocin and the L-analogue) deserves some comment. Fraser (1937) first observed that posterior pituitary extracts containing mainly oxytocin exert a bivalent effect on water diuresis in non-anaesthetized rats: in high doses, such extracts exhibit an antidiuretic activity in that the interval between the oral administration of the loading dose of water and the time of maximum rate of excretion is prolonged. But they also exert a diuretic action in that the total quantity of urine excreted during the period of enhanced diuresis is increased. It has recently been ascertained that both the antidiuretic and diuretic properties are inherent in the oxytocin molecule, for they are also shown by synthetic oxytocin which contains no vasopressin (Berde and Cerletti, 1956a). It is possible, however, that the diuretic activity of oxytocin is confined to one species only. Posterior pituitary extracts containing mainly oxytocin exhibit a diuretic activity in rats, not only in Burn's water diuresis test but also under various other experimental conditions (Fraser, 1937, 1942; Kuschinsky and Bundschuh, 1939; Schaumann, 1949; Brunner, Kuschinsky, Münchow, and Peters, 1956; Brunner, Kuschinsky, and Peters, 1956; Croxatto, Rosas, Zamorano, and Gonzalez, 1956). No diuretic effect has yet been observed in man (Brunner, Kuschinsky, Münchow, and Peters, 1956) or in dogs (van Dyke, Adamsons, and Engel, 1955), although it has recently been shown that in conscious dogs at resting rates of urine flow oxytocin elicits a marked increase in Na and Cl excretion (Brooks and Pickford, 1956).

The structural analogues of oxytocin included in these investigations possess in common several biological activities but to different degrees. It is evident that to characterize a compound with oxytocin-like properties a large number of tests must be used. The exclusive use of conventional bioassay methods for oxytocin, such as the avian blood pressure and the rat uterus *in vitro*, is not sufficient for this purpose.

In a recent paper, Ressler (1956) showed that the cyclic disulphide ring of oxytocin—deprived of the prolylleucyl-glycinamide side-chain—exhibited some effect on the isolated rat uterus and, to a smaller degree, on the mammary gland, whereas practically no influence on the chicken blood pressure could be detected. These results, like our own, emphasize the need to use a variety of tests to define the biological activities of compounds related to oxytocin.

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References

Berde, B., and Cerletti, A. (1956a). *Helv. physiol. acta*, **14**, 129.

—— —— (1956b). *Journées Franco-Belgo-Suisses de Gynécologie et d'Obstétrique, Bâle.* In the press.

Boissonnas, R. A., Guttmann, St., Jaquenoud, P. A., and Waller, J. P. (1955). *Helv. chim. acta*, **38**, 1491.

—— —— —— —— (1956a). Ibid., **39**, 1421.

—— —— —— —— Konzett, H., and Berde, B. (1956b). *Nature, Lond.*, **178**, 260.

Brooks, F. P., and Pickford, M. (1956). *Abstr. XXth int. physiol. Congr., Bruxelles*, p. 130.

Brunner, H., Kuschinsky, G., Münchow, O., and Peters, G. (1956). *Klin. Wschr.*, **34**, 451.

—— —— and Peters, G. (1956). *Arch. exp. Path. Pharmak.*, **228**, 457.

Burn, J. H. (1931). *Quart. J. Pharm.*, **4**, 517.

—— Finney, D. J., and Goodwin, L. G. (1950). *Biological Standardization*, 2nd ed. Oxford: University Press.

Clary, M. L., Cameron, A., and Craver, B. N. (1951). *Proc. Soc. exp. Biol., N.Y.*, **77**, 778.

Coon, J. M. (1939). *Arch. int. Pharmacodyn.*, **62**, 79.

Croxatto, H., Rosas, R., Zamorano, B., and Gonzalez, I. (1956). *Abstr. XXth int. physiol. Congr., Bruxelles*, p. 205.

Csapo, A. (1955). In *Modern Trends in Obstetrics and Gynaecology (Second Series)*, p. 20. London: Butterworth.

van Dyke, H. B., Adamsons, K. Jr., and Engel, S. L. (1955). *Recent Progr. Hormone Res.*, **11**, 1.

Fraser, A. M. (1937). *J. Pharmacol.*, **60**, 89.

—— (1942). *J. Physiol.*, **101**, 236.

Holton, P. (1948). *Brit. J. Pharmacol.*, **3**, 328.

Knaus, H. H. (1926). *J. Physiol.*, **61**, 383.

Konzett, H., Berde, B., and Cerletti, A. (1956). *Schweiz. med. Wschr.*, **86**, 226.

Kuschinsky, G., and Bundschuh, H. E. (1939). *Arch. exp. Path. Pharmak.*, **192**, 683.

Linzell, J L. (1955). *J. Physiol.*, **130**, 257.

Miller, L. C., and Tainter, M. L. (1944). *Proc. Soc. exp. Biol., N.Y.*, **57**, 261.

Ressler, C. (1956). Ibid., **92**, 725.

Rudinger, J., Honzl, J., and Zaoral, M. (1956). *Coll. Trav. chim. Tchécosl.*, **21**, 770.

Schaumann, O. (1949). *Experientia*, **5**, 360.

Schild, H. O. (1942). *J. Physiol.*, **101**, 115.

Thompson, R. E. (1944). *J. Pharmacol.*, **80**, 373.

du Vigneaud, V., Lawler, H. C., and Popenoe, E. A. (1953). *J. Amer. chem. Soc.*, **75**, 4880.

—— Ressler, C., Swan, J. M., Roberts, C. W., and Katsoyannis, P. G. (1954). Ibid., **76**, 3115.

—— —— —— —— —— and Gordon, S. (1953). Ibid., **75**, 4879.