ill—there is a high infection incidence of the young population, a large proportion of the cases being sub-clinical infections which can only be diagnosed by detection of virus in the blood, and a small proportion of frank cases with symptoms indicative of central nervous system infection. Thus, in the perpetuation and spread of this disease systemic infections probably have a more important role than actual clinical cases.

SUMMARY

Louping-ill, an encephalomyelitis of sheep, is caused by a filterable virus possessing neurotropic characters. Under natural conditions of infection the disease is tick-borne, the vector being *Ixodes ricinus* L. At first it would seem a curious anomaly that a blood-sucking arachnid has the role of transmitting a neurotropic virus, but the apparent anomaly is explained by the fact that multiplication of this so-called neurotropic virus occurs primarily in the blood. Invasion of the central nervous system by the virus occurs at a late stage in the infection, and is responsible for the characteristic symptoms of the disease. In the naturally occurring disease, invasion of the central nervous system is not a constant feature. Cases occur in which virus does not gain access to the central nervous system. Such abortive types of the disease can be diagnosed by the detection of virus in blood drawn at an early stage of the febrile reaction which accompanies the infection. It seems highly probable that the incidence of abortive types of the disease is greatly in excess of the number of frank cases with central nervous system involvement.

From a comparative point of view the study of louping-ill in sheep would suggest the possibility that in the early stage of poliomyelitis in man, before obvious clinical symptoms have developed, the specific virus may be present in the blood. If such early blood infection were demonstrated present views regarding the nature of poliomyelitis infection, its prevention, and treatment would require revision.

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URINARY EXCRETION OF OESTRIN ADMINISTERED UNDER EXPERIMENTAL CONDITIONS AND AFTER THE MENOPAUSE

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That oestrin is excreted by the human female in the urine and faeces at various stages during active sexual life is now definitely established. The quantities excreted show regular alterations during the menstrual cycle, these variations applying to the amounts found in both the urine and the faeces, and being greatly increased during pregnancy. A less marked increase in the oestrin excretion is also noted during the early stages of the menopause. The fluctuations in the elimination of oestrin correspond in general to changes in the hormone content of the blood. When, however, we come to consider the question as to how much oestrin is actually produced in the body at these different stages, and whether the hormone produced is quantitatively excreted without undergoing any changes, there are few experimental data on which any conclusions can be based. A similar paucity of information exists with regard to the function of the renal and intestinal barriers, and the problem of whether these play any part in determining the excretion of the sex hormone.

Zondek (1931) has, indeed, reported one case in which the elimination of oestrin was studied. The patient was oophorectomized for ovarian tumour, and subsequently the parenteral administration of 10,000 mouse units of oestrin was followed by excretion of 60 per cent. of the hormone in the urine within thirty-six hours. As some of the hormone was presumably also excreted in the faeces, the figures suggest that only a small percentage of the oestrin introduced in the body was retained and not excreted. On the other hand, both Siebke (1930) and Siebke and Schuschania (1930) also investigated the hormone excretion in the urine and faeces following the repeated administration of known doses both parenterally and hypodermically, and concluded that a large proportion of the hormone is *not* excreted, and must therefore, in some way, be used up or metabolized in the body.

OBJECT OF INVESTIGATION

Now knowledge as to the fate of the oestrous hormone is important for a number of reasons—namely:

1. It may help us to understand more perfectly the nature of the sex cycle and its relation to hormonal activity.
2. It will allow of a more exact interpretation of the diagnostic significance of data on the presence of the hormone in the excretions—in relation to the hormone production and activity.
3. It may help in the evaluation of the doses necessary to bring about certain desired therapeutic effects.
4. In view of the great variety of active oestrus-producing compounds now isolated, it may afford information as to which of these may be the most suitable in relation to the normal metabolic processes.

It was therefore decided to attempt to determine the fate of known quantities of a definite form of oestrin administered under strict experimental conditions. For the preliminary experiments it was deemed advisable to use subjects in which the normal oestrin production had presumably already ceased; and for this reason the experiments were all performed on patients in whom both ovaries had been surgically removed, or in whom the onset of the menopause had led to a cessation of the normal ovarian activity.

TECHNIQUE

The preparation used in these experiments was ketohydroxy-oestrin (obtained from B.D.H.). Samples were standardized by injecting four doses in oil over thirty-six hours into ovariectomized mice, and showed by this method a potency of about 5,000 mouse units per mg. The hormone was dissolved in absolute alcohol (1 mg. per c.cm.), and immediately previous to injection was diluted with four parts of water. Injections were in all cases given intramuscularly. The urine was collected for definite periods before and after injection. In the first experiment it was injected without treatment into the mice; this, however, proved rather toxic, and did not allow of a satisfactory estimation of the oestrin content.

RESULTS

As it was expected that the injection of the large doses of oestrin would be followed by the excretion of at least a substantial part of it, it appeared likely that standardization would be possible by means of the injection of the untreated urine. This expectation was not, however, realized. The procedure was adopted in the first case given in the table, but the whole of the urine passed during the first twelve hours following the injection contained less than 145 mouse units of the hormone (when standardization was performed by administration of four doses over thirty-six hours). Smaller amounts could not be detected, as the urine proved toxic when injected in the larger doses. Thus in this case, of the 5,000 mouse units of oestrin injected, less than 145 mouse units were excreted in the urine during the first twelve hours immediately following the administration.

In the next two cases the urine was not itself injected but extracted with ether, as described above under the heading "Technique." In both cases the ovaries had been previously removed, so that there could presumably be no question of any oestrin production by the patients.

Case No.	Age	Condition	Oestrin Content of Urine in 12 Hours Pre-injection Period	Amount of Oestrin Injected	Amount of Oestrin in Urine Following Injection								Material Assayed	Blood Assay	
					First 12 hours	Second 12 hrs.	Third 12 hrs.	Fourth 12 hrs.	Fifth 12 hrs.	Sixth 12 hrs.	Seventh 12 hrs.	Eighth 12 hrs.		Time After Oestrin Injection	Oestrin Content M.U. per Litre
I	57	Post-meno- pause (18 years)	M.U. —	mg. 1.0	M.U. <145	M.U. <140	M.U.	M.U.	M.U.	M.U.	M.U.	M.U.	Urine		
II	46	Bilateral ovari- ectomy	—	1.0	<30	24-72	>15						Ether extract of urine		
III	39	Bilateral ovari- ectomy	—	2.0	65	24-96						<10	Ether extract of urine		
IV	54	Post-meno- pause (10 years)	5-10	2.0	500-1,250	100-350				<30			Ether extract of hydrolysed urine	11½ hrs.	<25
V	52	Post-meno- pause (4-5 years)	>10	2.0	100				50				Ether extract of hydrolysed urine	4 hrs.	<25
					<30								Ether extract of urine		
VI	51	Post-meno- pause (7 years)	<9	2.0	30-100		<60						Ether extract of hydrolysed urine		

In later experiments the oestrin was therefore extracted by the following methods:

1. Portions of 270 c.cm. urine were acidified by the addition of 30 c.cm. 10 per cent. H_2SO_4 , and extracted with ether continuously for not less than eight hours in a continuous extractor. The ether extract was evaporated to dryness, and the residue dehydrated by several evaporations from solution in absolute alcohol. The dry residue was then dissolved in a mixture of ether and alcohol, and the requisite amount of maize oil added. The volatile solvents were removed by gentle heat, and the urine extract in the oily medium standardized.

2. In most instances the urine was boiled in acid solution for a number of hours before extraction with ether. Portions of 270 c.cm. urine were acidified with H_2SO_4 , as above, and the urine subjected to boiling under a reflux condenser for periods of about eight to twelve hours. The hydrolysate was then transferred to the continuous extraction apparatus and extracted with ether. The ether extract was treated as already described. Blood was also collected in two cases. The treatment of the blood samples was similar in every respect to the second process, described above for urine, except that the residue obtained on evaporation of the ether extract was dissolved in absolute alcohol and administered to the test animals in that form.

In all cases standardization was performed by the injection of four doses over thirty-six hours, the unit being taken as that amount which would produce cornification in 50 per cent. of the animals. In some cases, instead of attempting to obtain an exact figure, the amount of oestrin present in the sample was only determined within certain limits (see Table).

In the first of these cases 5,000 mouse units of oestrin (1 mg.) were given, and the second case received twice that amount. The amounts of oestrin excreted are shown in the table. It was found that the excretion of the hormone appeared again very small. Thus in Case III, which received about 10,000 mouse units (2 mg.), the extract made from the total urine collected during the twelve hours following the injection contained only about 65 mouse units of oestrin, while during the next twelve hours the urine contained (as determined by ether extraction) more than 24 mouse units and less than 96 mouse units (probably about 50 mouse units). Four days after the injection no more oestrin was detectable in the urine by this method.

The very small amounts of oestrin found in the urine in these two cases raised doubts as to whether all the hormone was being excreted in a form which could be extracted by ether. Dr. Marrian, from whom we inquired in regard to this question, kindly informed us that oestrin may be excreted in a form (probably a sulphate-ester) which is insoluble in ether, but which becomes soluble after hydrolysis. We thereupon performed the following experiment:

Two hundred and seventy c.cm. of urine obtained from a pregnant woman was extracted with ether for several hours. This ether extract was then dissolved in oil and standardized in the usual way, and was shown to contain definitely less than 120 mouse units of oestrin per litre of urine. The extracted urine was then hydrolysed for several hours with

H₂SO₄ and again extracted with ether. This extract was dissolved in oil and standardized. It contained more than 120 mouse units per litre but less than 600 mouse units per litre, the results showing that the actual amount was nearer the latter figure.

Hence, of the total amount of oestrin in this sample of urine only a small proportion (probably less than a quarter) was extractable by ether, the remainder becoming so only after hydrolysis.

In the next three cases, therefore, the urine was hydrolysed before extraction with ether. The excretion was, as in the previous cases, determined at various stages after injection, the dose administered being in all cases 2 mg. of oestrin—that is, about 10,000 mouse units. It will be noticed in the table that by this method the figures obtained are rather larger; yet only in one of these cases (Case IV) was the amount excreted at all large; for in the first twelve hours after the injection the urine contained about 1,000 mouse units of oestrin, but in the following twelve-hour period the urine contained a much smaller quantity of the hormone (more than 100 and less than 350 mouse units). By the third day after the injection the oestrin administered appeared to have been completely eliminated, as the urine contained only small amounts of the hormone. In the other two cases the proportion of injected oestrin which was excreted in the urine was even smaller, the total amount in the twelve hours following the injection being less than 100 mouse units.

In one of these cases (Case V) the proportions of oestrin excreted in ether-soluble and insoluble forms were investigated. A portion of the urine excreted during the first twelve hours following the injection was extracted with ether (without previous hydrolysis) and the extract standardized. It contained definitely less than 30 mouse units of oestrin per litre of the original urine. Another portion of the urine was first hydrolysed for several hours and then extracted with ether. This extract contained about 100 mouse units of oestrin per litre of the original urine. The residue of this extraction was further hydrolysed for a number of hours, and then again extracted with ether, but no oestrin could be detected in this extract. Hence, according to this experiment, less than one-third of the oestrin excreted in the urine was in an ether-soluble form (though the original material injected is ether-soluble).

Another interesting fact which emerged in the course of this investigation was that oestrin may be excreted in the urine several years after the onset of the menopause and cessation of menstruation, and in quantities apparently substantially similar to those present in the urine during the normal menstrual cycle. This was observed in two out of the three cases investigated. In Case V, a patient 52 years old, and investigated four to five years after the complete cessation of menstruation, the twelve-hourly sample collected before the injection of oestrin contained more than 10 mouse units of oestrin, corresponding to an excretion of the hormone of more than 20 mouse units per day. In Case IV, aged 54, and investigated ten years after the menopause, the excretion was about 10 to 20 mouse units per day. On the other hand, no appreciable amount of oestrin was detected in the urine of Case VI, aged 51, and investigated seven years after the menopause. In all these cases the urine was hydrolysed before extraction with ether.

An attempt was also made to detect oestrin in the blood after the administration, but in both cases investigated the extract obtained from 40 c.cm. of hydrolysed blood contained less than 1 mouse unit of the hormone. In the first case the blood was collected eleven and a half hours after the intramuscular injection of the hormone,

and this long interval might account for the small amounts of oestrin in the blood; but no such explanation can be offered in Case V, where the blood sample was taken four hours after administration of the oestrin.

DISCUSSION

The experiments reported clearly demonstrate that when ketohydroxyoestrin is injected into the human female subject only a small proportion of the hormone can be recovered in an active form from the urine by the methods used, which included not only thorough extractions with ether, but a similar extraction following prolonged hydrolysis. Moreover, the result is confirmed by the one experiment in which the urine was injected into ovariectomized mice without any previous treatment; for, although the toxicity of the urine rendered an accurate assay impossible, yet it could be definitely established that the quantities were comparatively small.

In view of the results of Siebke and Schuschania (1930), it is likely that some of the hormone was also excreted in the faeces, but, even allowing for this, only a portion of the amount injected can be accounted for. The proportion of the hormone excreted was not the same in all the subjects investigated. Assuming that the amount eliminated in the faeces is similar to that found in the urine, then the total amount of oestrin recoverable might, in Case IV, be equal to nearly one-quarter to one-third of the dose injected. In the other cases, however, and on a similar basis, it can be computed that less than one-twentieth of the hormone injected appears in the excreta. These results are, generally speaking, in agreement with those reported by Siebke (1930) and by Frank, Goldberger, and Spielman (1932) in the menstruating woman. They show, moreover, that even after cessation of menstruation—after the menopause or after the removal of the ovaries—the conditions are still substantially the same.

In view of these results it is difficult to escape from the conclusion that the human body can rapidly destroy, or at any rate render inactive, the oestrous hormone, and this view is supported by the finding that, four hours after the injection of 10,000 mouse units, the blood contained less than 25 mouse units of the hormone per litre (Case V). It may be mentioned that the evidence obtained by Fee, Marrian, and Parkes (1929) on the isolated heart-lung preparation also suggested that oestrin could be rapidly destroyed.

Further, the finding that a large amount of the hormone which appears in the urine is in a form not extractable by ether raises the question as to whether this portion represents an intermediate product in the inactivation of oestrin. In this connexion the results recently described by Silberstein, Engel, and Molnar (1933) are of great interest. These observers incubated oestrin with blood or liver tissue and found that by this method the hormone became rapidly destroyed or inactivated. Moreover, at one stage of this process they were still able to demonstrate the presence of a large proportion of the oestrin by the injection of the material into the ovariectomized animals; yet if this same material was extracted by means of an alcohol-acetone method a much smaller amount of oestrin was obtained. Later on, neither the material itself nor the alcohol-acetone extract contained any active oestrin. These results thus appear to show that *in vitro*, too, oestrin rapidly becomes inactivated, and that one of the intermediate products in the process of this inactivation is a form of the hormone not extractable by lipid solvents.

The finding that substantial amounts of oestrin may be excreted in the urine several years after the cessation

of menstruation appears highly interesting in relation to the genesis of the menstrual cycle. It is unlikely that the hormone found in the urine was ingested with the food, as comparatively large quantities of oestrin would need to be administered through the alimentary canal to give the rate of excretion observed. It must, however, be emphasized that in those post-menopausal cases in which oestrin excretion was found the urine was hydrolysed previous to the ether extraction, so that it is impossible to say whether any of the hormone originally present in the urine was in an ether-extractable form. This problem requires further investigation.

Lastly, the question arises as to the significance of the data so far obtained by a number of observers on the oestrin content of various body fluids and excretions. Where the material is directly injected into the ovariectomized mice (or rats), an assay of the total oestrous hormone content is presumably obtained. In those cases, however, where extraction of the material has been undertaken previous to the assay, results relating only to the hormone soluble in that particular solvent will be obtained, unless hydrolysis has also been performed. It may be urged that data relating to a definite form of oestrin (in so far as the solubility is concerned) are of more significance than the total quantity of oestrin-inducing hormone—that is, active in ovariectomized animals—present in a particular body fluid or excretion, and this may well be. On the other hand, the possibility that the rate of inactivation of the hormone may vary in different circumstances, physiological or pathological, and that the activity of the hormone in the body under normal or abnormal conditions may in part be determined by the rate of its inactivation, cannot be ignored, and further exploration of this aspect of the question appears to be necessary.

SUMMARY

Known doses of ketohydroxyoestrin have been injected intramuscularly into subjects, ovariectomized, or subsequent to the onset of the menopause, and the excretion of the hormone in the urine subsequently determined. It was found:

1. That only a small proportion of the oestrin administered could be recovered from the urine.
2. That a proportion of the hormone excreted was not extractable by ether. (This also applies to the oestrin excreted in normal pregnancy.)
3. That the blood of injected subjects contained less than one mouse unit of oestrin in 40 c.cm.

Incidentally it was also established that substantial amounts of oestrin may be excreted in the urine even eighteen years after the menopause.

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A CRITICISM OF ANTE-NATAL WORK*

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The genesis of ante-natal supervision as a recognized branch of preventive medicine appears to be associated with the name of the late J. W. Ballantyne, and to date from about the year 1902. Its value, however, can only be assessed with any pretence at accuracy over the last ten years, and, indeed, many competent judges question whether it has as yet received a fair trial. Be this as it may, it might with reason be expected that ante-natal supervision has been carried on to such an extent over this period of years that some results might by now be apparent in the figures of the national maternal mortality. Up to the present, however, no impression has been made on these figures, and if any change has occurred in the last few years the mortality has shown a slight rise. This fact has been a great disappointment, and has been the incentive for the production of papers of criticism and committees of inquiry. The published articles are not numerous, and, with one notable exception, devote themselves almost entirely to a debate upon the advantages and disadvantages to the community of the clinic system of ante-natal supervision.

SCOPE OF PRESENT REVIEW

In this criticism of ante-natal care an attempt will be made to review the actual work as it is being done to-day. If pre-natal supervision is unsatisfactory, or is actually wrong in its conception and execution, it matters not one jot or tittle whether it be performed by midwife, general practitioner, obstetric specialist, or by the medical men and women in charge of the twelve hundred or more ante-natal clinics that have appeared in the last few years.

It is assumed that all are in agreement that medical supervision of the woman in pregnancy is a most admirable thing, and constitutes a definite advance in medical science. Ballantyne in 1923, in a lecture at Nottingham, expressed his opinion that ante-natal care might be expected to result in the removal of the fear and dread of the confinement and of much anxiety and suffering, and also in the early and more satisfactory treatment of toxæmia, syphilis, and cardiac diseases associated with pregnancy, etc.

The aims and objects of this branch of preventive work are well expressed by Fairbairn in his textbook *Gynaecology with Obstetrics*, and read as follows:

"(1) To maintain the pregnant woman in health of body and mind.

(2) To preserve the pregnancy to its full time.

(3) To foresee and avert preventable difficulties and complications in labour and afterwards.

(4) To secure the best provision possible for the labour, prepare the woman for the nursing and care of her child, and educate her in the care of her own health and that of her family."

Fairbairn continues with the warning that "some enthusiasts have run the new thing to death, and their examinations have tended to make the patient think her condition pathological rather than physiological."

A consideration of the subject will be made upon the above lines. It is impossible to discuss all the illnesses that can complicate, or be complicated by, pregnancy, and therefore a selection has been made.

THE TOXAEMIAS

Toxic albuminuria occurs in about 5 per cent. of all cases. Apart from the immediate possibility of pre-eclampsia and eclampsia, it is only in recent years that the added danger of permanent renal damage following this condition has been demonstrated by Young,¹ Gibberd,² and others. While the death rate from toxic

* The substance of a British Medical Association Lecture given to the Norfolk Branch on April 18th, 1934.