

individuals seen in both 1958 and 1968 the numbers of women with P.C.V.s below 35% were 3 and 6 respectively; the numbers of men with P.C.V.s below 40% were 8 and 13 respectively. This increase in the number of subjects with low P.C.V.s in 1968 may be related to seasonal variation. Pucher *et al.* (1934), in four volunteers, found the highest P.C.V.s in late spring (the time of the 1958 survey) and some of the lowest levels in the autumn (the time of the follow-up in 1968). Clearly there are very considerable difficulties in determining any changes in prevalence in the distributions of haemoglobin or P.C.V.s.

Of 13 women below 12 g./100 ml. in 1958, three were again below 12 g./100 ml. in 1968 (Table VII). Of 12 men below 12.5 g./100 ml. in 1958, only one was again below that level in 1968. Using a stricter definition, Fry (1961) in a follow-up of anaemic cases in general practice for 5 to 10 years found that one-third were still anaemic. Beveridge *et al.* (1965) studied 378 hospital inpatients with hypochromic anaemia; on follow-up over a third of the women and over a quarter of the men were still iron-deficient. There is thus considerable evidence that once anaemia has been detected these subjects are more likely to be anaemic subsequently. The figures from the present survey suggest that this likelihood is increased in the selected

subjects identified in general practice (Fry, 1961) and in hospital patients (Beveridge *et al.*, 1965) compared with the unselected populations studied in this report.

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Steady-state Plasma Levels of Nortriptyline in Twins: Influence of Genetic Factors and Drug Therapy

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Summary: Nineteen identical (monozygotic) and 20 fraternal (dizygotic) sets of twins between 45 and 51 years of age were given nortriptyline orally in doses of 0.2 mg./kg. body weight three times daily for eight days. The steady-state plasma concentrations of nortriptyline were calculated from the mean of the determinations for days 6, 7, and 8. Identical twins, not treated with other drugs, achieved similar steady-state plasma concentrations of nortriptyline in contrast to fraternal twins who were not given other drugs. The intrapair similarity in steady-state plasma concentrations was not found in identical twins simultaneously treated with various drugs during the experiment. Identical and fraternal twins treated with drugs containing barbiturates had considerably lower steady-state plasma concentrations of nortriptyline compared with untreated twins.

It is concluded that most of the variability in nortriptyline steady-state plasma concentration between persons who have not received drugs is genetically determined. Exposure to other drugs also influences the steady-state plasma concentration of nortriptyline, which in a given patient may therefore be determined by a resultant of genetic and environmental factors.

Introduction

It has recently been established that patients who are treated with the same dose of various tricyclic antidepressants such as desipramine (desmethylinipramine) or nortriptyline show great differences in their steady-state plasma levels (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968). For desipramine (25 mg. t.i.d.) a range between 8 and 295 ng./ml. plasma has been observed (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968) and for nortriptyline, given in the same dose, steady-state plasma levels have been shown to vary fortyfold between patients (this laboratory, unpublished). Extensive pharmacokinetic investigations led to the suggestion that the steady-state plasma level of these drugs is mainly determined by their rate of metabolism, since only small individual differences were found in the plasma protein binding, degree of absorption, and renal elimination of these compounds in man (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968, 1969; Borgå *et al.*, 1969).

The metabolism of drugs in man may be influenced by both genetic (Evans, 1968) and environmental factors, such as exposure to other drugs (Levi *et al.*, 1968) and insecticides (Kolmodin *et al.*, 1969). In heavily drug-treated psychiatric patients it is impossible to evaluate the relative contribution of either factor to the quoted interindividual differences in steady-state plasma levels of tricyclic antidepressants.

This work was undertaken with a dual purpose: (a) to study the role of genetic factors in determining the steady-state plasma levels of nortriptyline, and (b) to assess the influence of concomitant drug therapy on this pharmacokinetic value.

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Material and Methods

Seventy-eight identical and fraternal twins, all between 45 and 51 years of age, and with one exception from the Stockholm area (monozygotic male, code 21, moved from Stockholm to Gotland in September 1968), voluntarily participated in this study (Table I a, b, and c).¹ Initially 105 pairs of twins were invited by letter through the Swedish National Twin Register, Solna, but for various reasons 66 pairs dropped out (17 not interested, 30 currently registered addresses incorrect or no answer to inquiry, 11 unable to participate because of occupation, 5 a member in hospital, and 3 dead).

TABLE II.—Analyses of Variance of Monozygotic "Not-Drug-Exposed" Twin Pairs

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
Between sexes	1	6.61	6.61
Within sexes	159	7,979.64	50.19
Between pairs within sexes	7	6,392.39	913.20
Within pairs within sexes	152	1,587.25	10.44
Between individuals within pairs	9	227.25	25.25
Within individuals within pairs	143	1,360.00	9.51
Between days within individuals	36	922.00	25.61
Within days within individuals	107	438.00	4.09
Total	160	7,986.25	49.91

- (A) $F_{(36;107)} = 25.61 \div 4.09 = 6.26$; $P < 0.0005$
 (B) $F_{(7;152)} = 25.25 \div 10.44 = 2.42$; $P > 0.05$
 (C) $F_{(7;152)} = 913.20 \div 25.61 = 35.66$; $P < 0.0005$
 (D) $F_{(1;159)} = 6.61 \div 50.19 = 0.13$; $P > 0.05$
 (E) $F_{(7;152)} = 913.20 \div 6.61 = 138.15$; $P > 0.05$

All subjects were Caucasian and with four exceptions healthy, as determined by history and physical examination, including electrocardiogram and routine laboratory analyses (haemoglobin, sedimentation rate, screening for protein and glucose in urine). The four exceptions were: (1) a man aged 50 (code No. 45) with cirrhosis of the liver diagnosed in 1967; laboratory studies (including haemogram, urine analysis, serum proteins, blood glucose, serum creatinine, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and thymol turbidity) showed normal results three weeks before the study; (2) a woman aged 51 (code No. 56) with grand-mal epilepsy; she was well controlled on diphenylhydantoin and phenobarbitone; (3) a woman aged 48 (code No. 57) with unconfirmed but drug-treated (isoniazid and para-aminosalicylic acid) tuberculosis; (4) a woman aged 51 (code No. 82) with thyroid carcinoma in 1958; she was well controlled on thyroid substitution therapy. From the clinical point of view there were no objections to include these four subjects in the study.

Each volunteer was typed for 12 different blood- and serum-group systems (ABO, MNS, P, K, Le, Fy, Jk, Rh, Gc, Hp, Gm, Ag) and red cell acid phosphatase, and the saliva was analysed regarding secretor or non-secretor to establish the nature of the twinship. Nineteen pairs were monozygotic and 20 dizygotic, in agreement with the records of the National Twin Register.

A careful drug history was obtained from all subjects. Twenty-one pairs did not use drugs for at least one year and were classified as "not exposed to drugs." In 18 pairs one or in some cases both twins were simultaneously treated with other drugs (Table I d). These were classified as "drug-exposed."

We also inquired about the living habits of the twins, with special emphasis on smoking and exposure to chemicals at home or at work.

Administration of Nortriptyline.—All subjects were given nortriptyline (5-(3-methylaminopropylidene)-10,11-dihydro-5H-dibenzo (a, d) cycloheptene chloride) 0.2 mg./kg. body weight by mouth three times daily for eight days. The drug was dispensed as gelatin capsules at the Military Pharmacy, Karolinska Hospital, Stockholm. Lactose was used as excipient. The capsules were taken at 6 a.m., 2 p.m., and 10 p.m.

¹ Copies of Table I, giving code numbers and details of all 39 pairs of twins, can be obtained from Dr. B. Alexanderson, Division of Clinical Pharmacology, Department of Pharmacology, Karolinska Institutet, Fack, S-104 01 Stockholm 60, Sweden.

Plasma Samples and Analysis of Nortriptyline.—Blood specimens were drawn between 11 a.m. and 1 p.m. on days 6, 7, and 8 of drug therapy and placed in heparinized tubes. The blood was centrifuged for 10 minutes at 2,000 r.p.m. Then 6 ml. of plasma was removed and mixed with 1.5 ml. of 0.1 N HCl. The plasma samples were stored at -20° C. and later assayed for nortriptyline according to the method of Hammer and Brodie (1967) as described by Sjöqvist *et al.* (1969). All specimens were coded, the analyst being kept unaware of the relationship between the twins and about their classification as "drug exposed" or "not drug exposed." Each plasma sample was analysed in triplicate with five exceptions (Table I b and c). The steady-state level used for calculation was the mean of the concentrations on days 6, 7, and 8.

Statistical Methods.—The statistical analysis of the variances is based on a nested classification according to Snedecor and Cochran (1967). The total variation was divided into component sums of squares corresponding to the variation between and within sexes, pairs, individuals, and days (Tables II, III, and IV).

Results

Methodological Error.—The variance within days within individuals represents the methodological error. There is a small difference of the variance within days within individuals between not-drug-exposed dizygotic and monozygotic twins; $F_{(143;107)} = 5.73/4.09 = 1.40$; $0.05 > P > 0.025$ (Tables II and III). So far we cannot explain this. The methodological error should be equal in drug-exposed and not-drug-exposed twins provided that simultaneous treatment with other drugs does not interfere with the analysis of nortriptyline. In two subjects (code Nos. 63 and 65) treated with dextropropoxyphene chloride (Doloxene) during the experiment the plasma blanks diverged from those of the respective sib. This compound was found to interact with the analysis of nortriptyline. Both the involved twinships were excluded from the calculations.

TABLE III.—Analyses of Variance of Dizygotic "Not-Drug-Exposed" Twin Pairs

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
Between sexes	1	437.27	437.27
Within sexes	213	36,487.69	171.30
Between pairs within sexes	10	25,472.96	2,547.30
Within pairs within sexes	203	11,014.73	54.26
Between individuals within pairs	12	9,133.62	761.14
Within individuals within pairs	191	1,881.11	9.85
Between days within individuals	48	1,061.78	22.12
Within days within individuals	143	819.33	5.73
Total	214	36,924.96	172.55

- (F) $F_{(48;143)} = 22.12 \div 5.73 = 3.86$; $P < 0.0005$
 (G) $F_{(12;48)} = 761.14 \div 22.12 = 34.41$; $P < 0.0005$
 (H) $F_{(10;213)} = 2,547.30 \div 171.30 = 14.87$; $0.05 > P > 0.025$
 (I) $F_{(1;213)} = 437.27 \div 171.30 = 2.55$; $P > 0.05$
 (K) $F_{(10;213)} = 2,547.30 \div 437.27 = 5.82$; $P > 0.05$

TABLE IV.—Analyses of Variance of Monozygotic "Drug-Exposed" Twin Pairs

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square
Between sexes	1	19,797.74	19,797.74
Within sexes	157	34,635.75	220.61
Between pairs within sexes	7	27,786.42	3,969.49
Within pairs within sexes	150	6,849.33	45.66
Between individuals within pairs	9	5,418.93	602.10
Within individuals within pairs	141	1,430.40	10.14
Between days within individuals	35	859.73	24.56
Within days within individuals	106	570.67	5.38
Total	158	54,433.49	344.52

- (L) $F_{(35;106)} = 24.56 \div 5.38 = 4.57$; $P < 0.0005$
 (M) $F_{(9;157)} = 602.10 \div 22.06 = 27.29$; $P < 0.0005$
 (N) $F_{(7;157)} = 3,969.49 \div 602.10 = 6.59$; $0.01 > P > 0.005$
 (O) $F_{(1;157)} = 19,797.74 \div 3,969.49 = 4.99$; $P > 0.05$

The total degrees of freedom are not equal in Tables II and IV despite the numbers of twinships being equal in Figs 2 and 4. This is because of a few missing determinations. In the complete experiment five plasma samples could be processed in duplicate only (and not in triplicate); and for one subject there was no plasma sample for day 8. (For details see Table I.)

Interindividual Differences in Steady-state Plasma Levels of Nortriptyline in Entire Material.—Fig. 1 shows the inter-individual differences in steady-state plasma concentrations of nortriptyline in both drug-exposed and not-drug-exposed twins. The range between the extremes is tenfold (8–78 ng./ml. plasma).

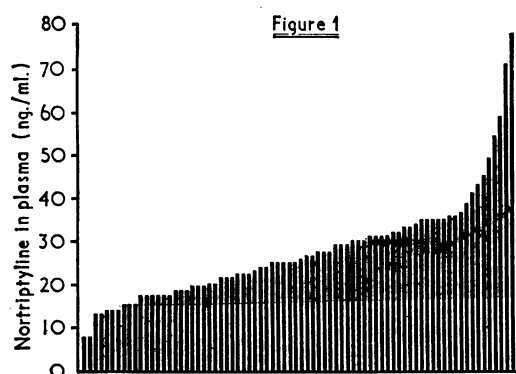


FIG. 1.—Histogram of entire material except for two subjects, code Nos. 63 and 65 (see Table Ic). FIG. 2.—Mean nortriptyline plasma concentration of days 6, 7, and 8 in identical twins previously not exposed to drugs. FIG. 3.—Mean nortriptyline plasma concentration of days 6, 7, and 8 in fraternal twins previously not exposed to drugs.

Not-drug-exposed Twins.—Monozygotic twins, previously not treated with other drugs, achieved practically the same steady-state plasma concentration of nortriptyline. The individual steady-state levels varied from 14 to 35 ng./ml. plasma (Fig. 2). The analysis of variance did not indicate any differences in the steady-state plasma concentrations between individuals within the pairs (F-ratio (B), $P > 0.05$). There were significant differences between the pairs (F-ratio (C) $P < 0.0005$) (Table II). Dizygotic twins not taking other drugs (Fig. 3) showed significant intrapair (F-ratio (G), $P < 0.0005$) and interpair (F-ratio (H), $0.05 > P > 0.025$) differences in steady-state plasma concentrations of nortriptyline (Table III).

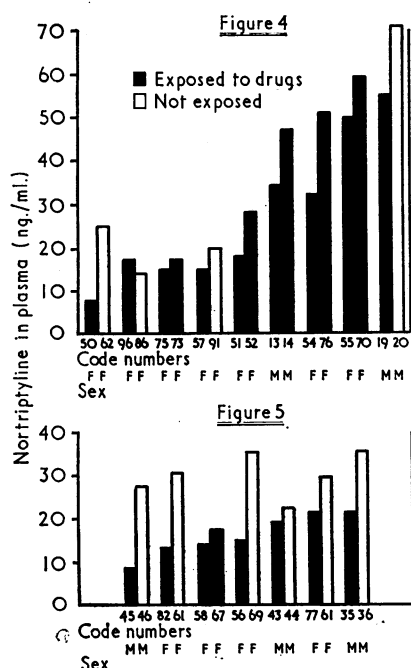


FIG. 4.—Mean nortriptyline plasma concentration of days 6, 7, and 8 in identical twins exposed to different drugs. FIG. 5.—Mean nortriptyline plasma concentration of days 6, 7, and 8 in fraternal twins exposed to different drugs.

Drug-exposed Twins.—The intrapair similarity in steady-state plasma concentrations was *not* found in monozygotic

twins who were on treatment with various drugs during the experiment (Fig. 4). The statistical calculations revealed significant differences in steady-state plasma concentrations of nortriptyline both between individuals within the pairs (F-ratio (M), $P < 0.0005$) and between the pairs (F-ratio (N), $0.01 > P > 0.005$) (Table IV). Fig. 5 shows the steady-state plasma

concentrations of nortriptyline found in drug-exposed dizygotic sets of twins. Fraternal and identical twins treated with various drugs containing barbiturates had considerably lower steady-state plasma concentrations of nortriptyline than the untreated control twins (code Nos. 50 + 62, 19 + 20, Fig. 4; code Nos. 56 + 69, 43 + 44, 77 + 66, Fig. 5). The monozygotic twins (code Nos. 73 and 75), both of whom were on drugs containing barbiturates, had similar but comparatively low (17 and 15 ng./ml. respectively) steady-state plasma levels of nortriptyline (Fig. 4).

There was no significant correlation in this study between smoking habits (cigarettes per day) and the steady-state plasma concentration of nortriptyline (Table I a, b, and c); $r = -0.1526$; $F_{(1,48)} = 1.14$; $P > 0.05$. There were no differences between sexes in steady-state plasma levels (F-ratios (D), (E), (I), (K), and (O), $P > 0.05$).

The steady-state plasma concentrations of nortriptyline differed significantly between the days within the individuals (F-ratios (A), (F), and (L), $P < 0.0005$). This may be expected because the steady-state plasma concentration fluctuates to a small extent in relation to the dosage interval. As mentioned above, the plasma samples were taken any time between 11 a.m. and 1 p.m.

Discussion

After repetitive oral administration of a constant dose of nortriptyline at fixed intervals the plasma concentration of the drug increases successively until a steady state is achieved. In agreement with earlier findings in patients on treatment with nortriptyline (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968) our results show that healthy adults develop different steady-state plasma concentrations when treated with the same dose of nortriptyline per kilogram body weight.

This twin study shows that most of the variability in nortriptyline steady-state plasma concentration between not-drug-treated individuals is genetically determined. All identical twins lived apart in different households; therefore the close resemblance in steady-state plasma concentrations of nortriptyline cannot be accounted for by exposure to common environmental factors.

It is known that individual variations in absorption, plasma protein, and tissue binding, rate of metabolism, and renal elimination may affect the steady-state plasma concentration of

a drug. Therefore it is important to unravel the possible pharmacological factors involved. Monomethylated tricyclic antidepressants are easily absorbed from the intestine (Bernhard and Beer, 1962; Hermann, 1963; Yates *et al.*, 1963; Sjöqvist and Borgå, 1969, unpublished). The renal excretion of unchanged nortriptyline has been shown to account for less than 5% of the daily dose at a steady-state plasma concentration in man (Sjöqvist *et al.*, 1968, 1969). The interindividual differences in binding of desipramine and nortriptyline to plasma proteins are negligible (Borgå *et al.*, 1969) compared with the observed variations in steady-state plasma concentrations. This may not necessarily exclude some interindividual differences in tissue binding.

Under steady-state conditions the amount of absorbed drug should be equal to the amount eliminated. The plasma concentration is kept almost constant in equilibrium with the body tissues. For these reasons the steady-state plasma concentration seems to be generally a much more accurate pharmacokinetic value than, for example, the plasma half-life following a single oral dose. The latter value is more dependent on fluctuations in rates of absorption and distribution. If, however, the drug is discontinued after steady-state has been established the plasma half-life will then more accurately reflect the rate of elimination, since the tissues are equilibrated with the drug (Hammer *et al.*, 1967). Vesell and Page (1968a, 1968b, 1968c) reported that the pronounced differences between individuals in antipyrine, phenylbutazone and dicoumarol metabolism are primarily under genetic rather than environmental control, taking the plasma half-life of a single oral dose as an index of drug metabolism.

According to van Rossum and Tomez (1968) and van Rossum (1968) the steady-state plasma concentration (C) of a drug is directly proportional to the maintenance dose (Q) and half-time for elimination ($t_{1/2}$) and is inversely proportional to the dosage interval (Δt) and the "volume of distribution" (V) as to be expressed mathematically:

$$C = 1.44 \frac{Q \cdot t_{1/2}}{V \cdot \Delta t}$$

van Rossum and Tomez (1968) also reported that this formula is satisfied by the data on steady-state kinetics of desipramine in man obtained in this laboratory (Hammer and Sjöqvist, 1967).

The volume of distribution (V) is the ratio of the concentration of drug in the body (mg./kg.) to the concentration in plasma (mg./l.) and hence an index of accumulation in body tissues. V is readily computed from the decline in plasma concentrations after parenteral administration of a known amount of drug (Brodie and Reid, 1967). If the maintenance dose and the dosage interval are kept constant interindividual variations in steady-state plasma concentrations will be due to differences in $t_{1/2}$ and/or V , provided that absorption of the drug is complete. It will be of interest to try in the future to evaluate the relative importance of both volume of distribution and rate of metabolism in regulating the steady-state plasma concentration of nortriptyline.

None of the kinetic values for nortriptyline discussed so far except $t_{1/2}$ seems to involve active enzymatic processes. The pronounced interindividual differences in steady-state plasma levels of nortriptyline could be explained by corresponding differences in the metabolism of the compound. It may be possible to identify genetic polymorphisms in the activity of enzymes controlling individual chemical biotransformations. Recent cross-over studies show that psychiatric patients treated with the structurally related compounds nortriptyline and desipramine achieve similar plasma concentrations on both drugs (Hammer *et al.*, 1967).

Nortriptyline is metabolized by hydroxylation, desmethylation, and conjugation with glucuronic acid in the rat (McMahon *et al.*, 1963) and in man, where hydroxylation seems to be the major metabolic pathway (Hammer, and Sjöqvist, unpublished data).

From extensive animal investigations it is well known that phenobarbitone and other barbiturates may accelerate the metabolism of drugs by increasing the activity of hydroxylating enzymes in liver microsomes (cf. Burns and Conney, 1965).

In two identical and three fraternal sets of twins one of the sibs respectively was on concomitant treatment with various drugs containing barbiturates. All these individuals had considerably lower steady-state plasma levels of nortriptyline than the untreated control twins. In one monozygotic pair (code Nos. 73 and 75) *both* individuals were on barbiturate treatment. These twins not only achieved low steady-state levels of nortriptyline (17 and 15 ng./ml. plasma) compared with the total range in steady-state concentrations (8–78 ng./ml. plasma) but they also showed a remarkable intrapair similarity.

So far it is not clarified whether barbiturates or other drugs may interfere with the absorption of tricyclic antidepressants or change their volume of distribution. Regarding the latter, it is possibly relevant that phenobarbitone does not affect the binding of desipramine to plasma proteins (Borgå *et al.*, 1969). These facts suggest that the steady-state level of nortriptyline is decreased in barbiturate-treated twins owing to induction of microsomal drug-metabolizing enzymes.

Only two individuals asserted exposure to chemical agents at home or at work. These two (code Nos. 13 and 14)—monozygotic "drug-exposed" males—are in close contact with gases and different chemicals in their daily work as welders.

Our data show that, besides genetic factors, concomitant drug exposure is fundamentally important in determining the steady-state plasma levels of nortriptyline. Thus the intrapair similarity in steady-state level of nortriptyline was not found in monozygotic twins who were treated with other drugs during the experiment.

This finding has clinical implications in relation to the extensive drug combinations commonly used in the treatment of psychiatric disorders. Fixed combinations of antidepressants and potential inducers of drug metabolism are often used. Probably drug interactions have not been recognized in clinical psychopharmacology owing to the difficulties in assessing both therapeutic and adverse effects quantitatively. Accumulating evidence indicates that the pharmacological effects of nortriptyline are directly related to its steady-state plasma concentration (Freyschuss *et al.*, 1969, and unpublished data). It therefore becomes important to recognize factors determining this pharmacokinetic value. In this regard nortriptyline may be looked on as a model for a great number of currently used drugs.

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Human Babesiosis in Ireland: Further Observations and the Medical Significance of this Infection

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[WITH COLOUR PLATE]

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Summary: Three splenectomized persons in Yugoslavia, California, and Ireland have been reported to be infected by three different *Babesia* species; two cases were fatal. In a study of the site where the fatal infection was contracted in Ireland, blood samples from 36 persons who had recently been bitten by ticks were inoculated into two splenectomized calves; no response to *Babesia divergens* was detected. Field-collected *Ixodes ricinus* ticks inoculated into another splenectomized calf resulted in fever and recovery of the agent of tick-borne fever (*Cytoecetes phagocytophilia*). This attempt to determine the presence of latent infection in human beings with intact spleens should be repeated on a larger scale in areas with a demonstrably high incidence of *Babesia* in ticks and animals. Few places in the world are free of piroplasms; their presence may present a hazard to splenectomized persons or to those whose splenic function is deficient.

Introduction

Three human cases of babesiosis have now been reported in the literature. Each occurred in a person who had undergone splenectomy some time previously, and was severe or fatal. Human beings with an intact spleen are apparently insusceptible to this cosmopolitan protozoal infection of the lower vertebrates.

The first human case was reported from Yugoslavia (Škrabalo and Deanovic, 1957). A farmer living on the outskirts of Zagreb had suffered internal injuries in a car accident 11 years

earlier and his spleen had been removed. He remained well until June 1956, when he was admitted to hospital very ill with fever, anaemia, jaundice, and haemoglobinuria. His blood contained numerous intraerythrocytic "rings" (Colour Plate, Fig. 1) which were identified as *Plasmodium falciparum* (Colour Plate, Fig. 2), and the condition was thought to be blackwater fever. The patient died eight days later. The rather unusual characters of the case raised some suspicion about the correctness of the diagnosis, and the blood films were sent for confirmation to Drs. N. H. Swellengrebel, R. Geigy, and E. Mudritz, and to one of us (P. C. C. G.). Each replied that the infection was babesial, not malarial. It seemed possible that the slides had been muddled with veterinary ones, but corpuscle measurements quickly confirmed that the cells were of human and not bovine dimensions. Epidemiological inquiries showed that the farm where the man lived was infested by ticks (*Dermacentor silvarum* and *Ixodes ricinus*) and that the cattle he had tended were infected with *Babesia bovis*.

The second case occurred in California in 1966 in a man who had visited relatively isolated coastal areas near San Francisco during the months preceding illness. He was admitted to hospital with chills and fever, diagnosed as a case of malaria, placed on 250 mg. of chloroquine weekly for about 15 weeks, and recovered (Scholtens *et al.*, 1968). Because of hereditary spherocytosis, a splenectomy had been performed on the patient in 1964, at which time he received 13 units of blood. The case was diagnosed as babesiosis (*Babesia* sp.) when "Maltese-cross"-type organisms, but no malarial parasites, were found on the blood slides. Animal inoculations with blood taken about 90 days after onset of illness demonstrated no organisms. *Babesia* antibodies in the patient's serum were found in both complement-fixation and tube latex-agglutination tests.

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§ But see Fitzpatrick *et al.* (1969) regarding a newly described fourth instance of human babesiosis.

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