Journal of Pharmaceutical & Biomedical Analysis
Vol. 1, No. 3, pp. 247–257, 1983
Printed in Great Britain

0731–7085/83 $3.00 + 0.00

Analytical Survey

Drug effects on clinical laboratory tests

G. SIEST[1,2,3]*, S. J. DAWKINS[3] and M. M. GALTEAU[1,2,3]

[1] *Centre du Médicament, Université de Nancy I, 7 rue Albert Lebrun, 54000 Nancy, France*
[2] *Centre de Médecine Préventive, Avenue du Doyen Jacques Parisot, 54500 Vandoeuvre-les-Nancy, France*
[3] *Faculté des Sciences Biologiques et Pharmaceutiques, Laboratoire de Biochimie Pharmacologique, Université de Nancy I, 7 rue Albert Lebrun, 54000 Nancy, France*

Abstract: Drugs and xenobiotics can affect clinical laboratory test results either by interfering with the analytical systems themselves, or by influencing endogenous constituents. National and international bodies have brought widespread recognition to this problem and have proposed protocols for its thorough scientific study. In this survey the authors discuss studies in their laboratories concerning the effects of drugs on thousands of patients undergoing a routine clinical screen. A database is described for storing both patient information and a detailed analysis of the published literature on drug effects.

Analytical interferences in clinical tests must be examined in validating the procedure. However, highly specific analytical techniques are increasingly helping to reduce such interferences. Biological effects can be classified as physiological, pharmacological or toxicological. In some cases, biological effects can be used to advantage in monitoring treatment by potentially hazardous drugs, such as the cardiac glycosides. The requirement for a well-defined reference population for each drug and for access to all clinical and medical data for each patient is discussed. The need for greater awareness of the influence of drugs on clinical laboratory results is considered, together with the suggestion that the health professions should try to exploit such effects in monitoring possible toxicity problems, in defining genetic constitution and in designing medication programmes.

Keywords: *Drug therapy; therapeutic drug monitoring; analytical interference; clinical chemistry; database on clinical and analytical effects; oral contraceptives.*

Introduction

Clinicians, clinical chemists, and pharmacologists and indeed all those working in the field of pathology are becoming increasingly aware of the effects of drugs on clinical laboratory tests. Such effects can nevertheless pass unnoticed, since laboratory tests are frequently requested independently of drug treatment. Clinical laboratory tests play an ever increasing role in monitoring drug therapy. Until recently they were used primarily

* To whom correspondence should be addressed.

in assessing the efficacity of hypoglycaemic, hypolipaemic or anticoagulant therapy. Nowadays such tests also find use in liver or kidney examination, or in the exploration of calcium and phosphorus metabolism in subjects undergoing tranquillizer, hypnotic or oral contraceptive therapy. Laboratory tests such as those for triglycerides have recently gained recognition as a means of assessing oral contraceptive treatment. Other clinical laboratory tests are also being used to monitor treatment by drugs known to cause enzyme induction. The causes of any changes observed in endogenous components must be clearly defined, since such variations could be due to factors other than drugs, including preanalytical variation, the age, sex, weight of the subject, etc.

One of the problems in interpreting the effects of drugs on clinical laboratory tests is that the growing literature currently provides insufficient essential information. Consequently, expert groups have been established within the European Economic Community, (DICC), and also under the auspices of the International Federation of Clinical Chemistry (IFCC, Scientific Committee — Clinical Section, on 'drug effects in clinical chemistry'). These groups aim to prepare protocols and rules to define a clear, scientific approach in solving the problems involved (M.M. Galteau and G. Siest, 'Drug Effects in Clinical Chemistry', in preparation). The objectives of the IFCC Expert Panel are:

1. To consider interference with analytical procedures caused by chemical or physical properties of drugs;
2. To determine the *in vivo* effects of drugs;
3. To describe the influence of drugs on clinical reference limits;
4. To select clinical laboratory tests for drug trials;
5. To evaluate clinical laboratory tests used in monitoring treatment and in predicting drug intolerance.

At the national level in France, similar work is being undertaken by the Société Française de Biologie Clinique (SFBC) [1, 2]. These three bodies make use of information available internationally, together with a considerable amount of work performed in the laboratory of the Centre for Preventive Medicine, Vandoeuvre-les-Nancy and in the Biochemical Pharmacology Department of the University of Nancy.

In order to clarify information found in the literature, a database on the effects of drugs on laboratory tests has been established at the Centre for Preventive Medicine [3]. It is the third such database to be compiled, others being in Sweden, under the direction of N. Tryding, and the United Kingdom under the direction of J.G. Salway. The database in Nancy also contains information on any drug effects recorded during the routine screening of patients attending the Centre for a check-up.

Drug effects on clinical laboratory tests classified as: purely analytical, where the drug and/or its metabolite or metabolites could influence the analysis of a component at some stage of the analytical procedure; or biological, where the drug and/or its metabolite or metabolites could be responsible for the modification of a biological component by means of a physiological, pharmacological or toxicological mechanism. This latter category includes unexpected secondary effects, desirable or otherwise.

Analytical Interference

Analytical interferences are one aspect of the general effects of drugs on clinical laboratory tests. However, they account for only about 20 per cent of the publications cited by Young *et al.* [4]. Such interferences can be attributed to the drug itself and/or to

its metabolite or metabolites. Physical properties such as colour or fluorescence of the drug are sometimes responsible and are especially important when reactions are performed under strongly acid or alkaline conditions. Chemical interferences are generally more important when high drug concentrations are involved. Reduction of the drug and/or its metabolites is often a significant factor; thus ascorbic acid interferes in many analyses, thanks to its marked reducing properties [5], as illustrated in Fig. 1. Other possible chemical interferences include complex formation and precipitation.

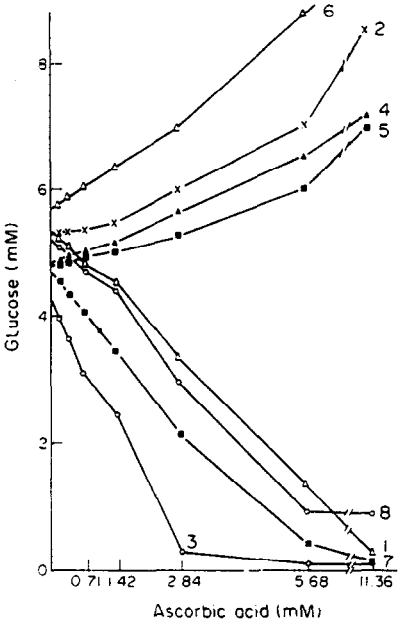

Figure 1
Interference by ascorbic acid with respect to different techniques for glucose measurement [5]. 1, Glucose oxidase–perid method (Vitatron UC 200 S and Digilog DRP 200 methods); 2, 4, 5, 6, Neocuproin (Technicon SMA 12/60 method); 3, Glucose oxidase–perid (Technicon method); 7, Glucose oxidase–peroxidase (LKB 7400 method); 8, *Trinder* method, glucose oxidase (Gilford method).

More specifically, drugs can influence the measurement of enzyme activities and protein concentrations by blocking or modifying certain catalytic or immunochemical sites used in their analysis. This type of interference generally leads to inhibition, but occasionally there is activation of the corresponding protein.

It might appear from the foregoing that analytical interferences pose important problems in the clinical laboratory. Fortunately, however, clinical methods are continually being improved to give greater specificity and precision, thereby reducing the risk of this type of interference. Nevertheless, it is vital to define assay protocols for each new method, bearing in mind that existing methods are sometimes simplified by adapting them to new processes or equipment, potentially to the detriment of specificity [2]. A DICC study group sponsored by the EEC [5] has demonstrated that enzymatic methods do not necessarily obviate interferences, as illustrated by the data on glucose assays in Fig. 1. Ascorbic acid interferes with the glucose oxidase–peroxidase method by affecting the peroxidase catalysed redox reaction. Thus in this two-step enzymatic method the specificity achieved in the first glucose oxidase step is negated by the lack of specificity in the subsequent peroxidase stage.

Following the work of the European Study Group, both the SFBC Commission and the IFCC Expert Panel on 'Drug effects in clinical chemistry' undertook to prepare well-defined protocols for the study of drug interferences [2]. It is the aim of the two groups to introduce a degree of uniformity in protocols for testing for potential drug interferences,

so that results may be more readily comparable from one laboratory to another. It is hoped that these recommendations would be adopted both by reagent manufacturers, for testing new analytical methods, and by pharmaceutical companies, for testing new drugs. The protocols define, for example, drug solubilization in aqueous media, the nature of the specimen to be spiked with the drug (fresh or frozen drug-free human serum), the drug concentration to be tested, initially taken to be ten times the maximum therapeutic serum concentration or, if that is unknown, five times the maximum daily dose.

Pre-analytical Interference

Pre-analytical interferences take into account all sources of variation occurring from the moment when the blood or other biological sample is taken, until the sample is introduced into the sequence of analytical events. Such variations can be an important source of error. Until recently the problem of pre-analytical interference has been largely ignored, although Statland *et al.* [6] and others have pointed out its importance.

Greenblatt *et al.* [7] have shown that the administration of drugs intramuscularly just before taking a blood sample leads to error in the measurement of serum creatine phosphokinase (CPK) activity. Figure 2 shows the type of serum CPK increase observed 4 h after an intramuscular injection [7]. In this case the drug has little effect; rather it is the injection solvent medium *per se* which leads to the increase observed in enzyme activity.

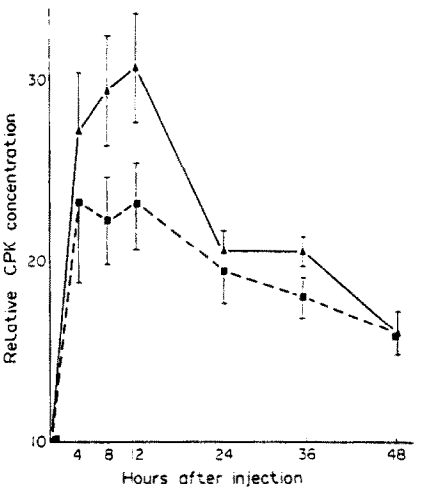

Figure 2
Relative serum creatine phosphokinase activity 4 h after an i.m. injection of 50 mg chlordiazepoxide chlorhydrate in 12 subjects (▲) or of the injection solvent alone in 9 subjects (■) [7].

Drug Effects

In order to assess drug effects on clinical laboratory tests, it has become customary to use the publication of Young *et al.* [4], although this work can, perhaps, be considered to be limited, since the amplitudes of the effects are not given. The National Corporation of Swedish Pharmacies [8] has gone some way towards providing this important information insofar as the effects cited are expressed as a percentage of the subjects studied. The following examples are intended to illustrate some aspects of the important problems in the area of drug effects on clinical tests.

Drug effects on plasma gamma-glutamyltransferase. The activity of the plasma enzyme gamma-glutamyltransferase (GGT) has been shown to be a sensitive index of drug metabolism; it is affected positively or negatively according to drug class (Fig. 3) [9]. Thus, hypolipaemic agents have no effect in females but lead to a 24% decrease in plasma GGT activity in males. Uricosuric drugs, on the other hand, exert a greater effect in women (26% increase in GGT activity) than in men. Likewise, antianginal agents have no effect in men but lead to a 41% increase in plasma GGT activity in women. Anticonvulsant therapy exerts the most marked effect, increasing plasma GGT activity by about 200% in both sexes.

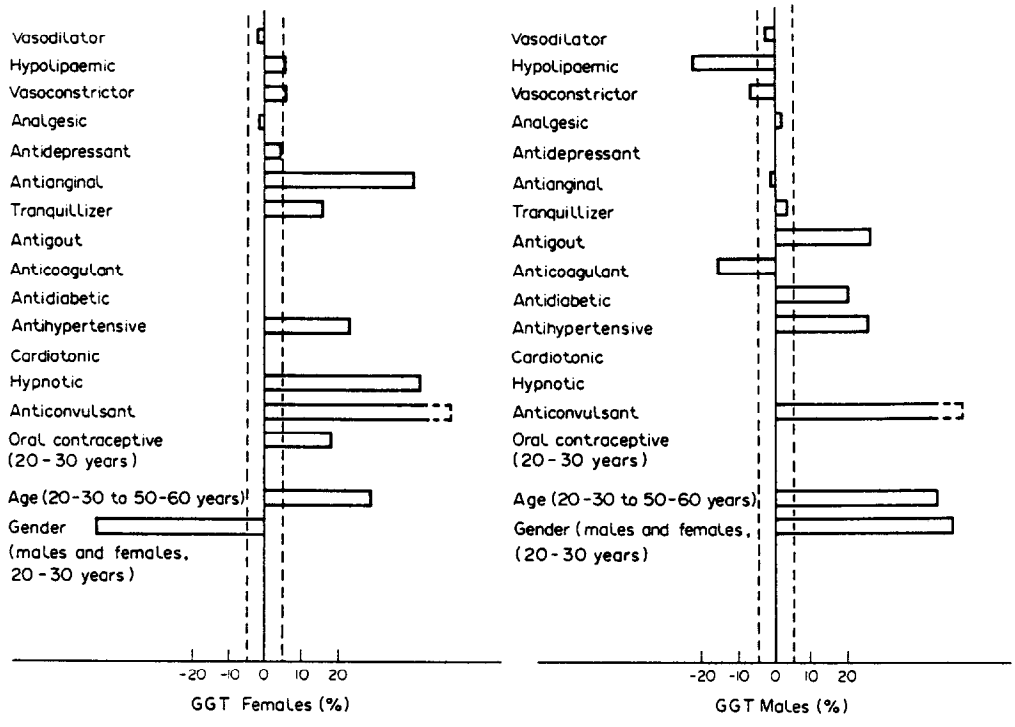

Figure 3
Effects of long-term drug therapy on plasma gamma-glutamyltransferase activity in males and females in the 40–60 year age group (expressed as percentage of the median) [9].

There is strong evidence that it is essential to know the precise duration of drug treatment. Thus the increase in plasma enzyme activity in women taking oral contraceptives differs as between those who have taken this treatment for less than 6 months, and those who have taken it for one or two years (Figs 4 and 5) [10]. These effects also vary with age and other biological variables. For this reason it is believed by the authors that any subject undergoing drug therapy must be excluded from populations used in the establishment of reference values.

Drug monitoring. The accumulated results of several years have led to a greater understanding of the significance of drugs amongst the other biological variables [11, 12]. In preventive medicine risk factors are of special interest, among which can be included

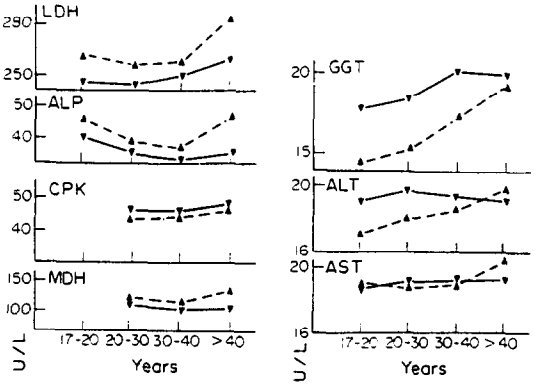

Figure 4
Variations in plasma enzyme activities with age in women taking oral contraceptives [10]: LDH, lactate dehydrogenase; ALP, alkaline phosphatase; CPK, creatine phosphokinase; MDH, malate dehydrogenase; GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; (———) treated; (– – –) controls.

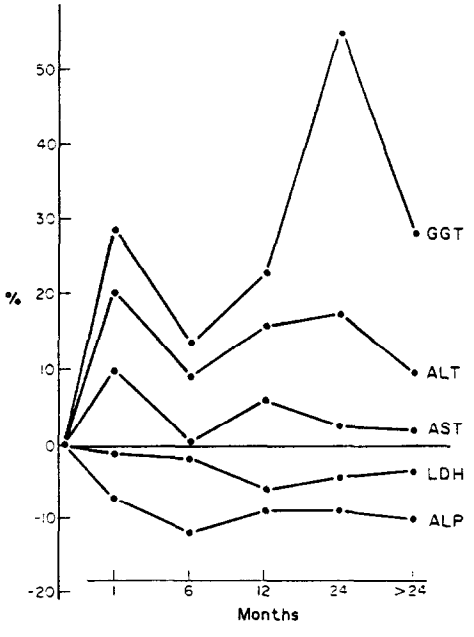

Figure 5
Percentage variation in plasma enzyme activities of women in the 20–30 year age group, with duration of oral contraceptive treatment [10]; abbreviations as in legend to Fig. 3.

drugs administered long-term, such as anticonvulsants, oral contraceptives, tranquillizers, antihypertensives and hypolipaemic agents. The effect of oral contraceptives on the accepted limits of plasma triglycerides in women exemplifies this point (Fig. 6). The shift in the median and extreme centiles is of the same magnitude, thus enabling the group of women taking contraceptives to be defined, since their reference values are functions not only of physiological factors but also of the type of steroidal drug being taken. This clear shift in plasma triglyceride levels permits decision limits to be established for stopping or changing the steroidal drug. Indeed, if the plasma triglyceride level before therapy were known, together with the mean of any bias introduced into the test by oral contraceptives (by comparison with a reference population of women taking the same steroidal formulation), the clinician would be able to interpret more accurately the triglyceride levels measured after several months' medication. It is clearly necessary, therefore, for clinicians to be kept informed of such effects.

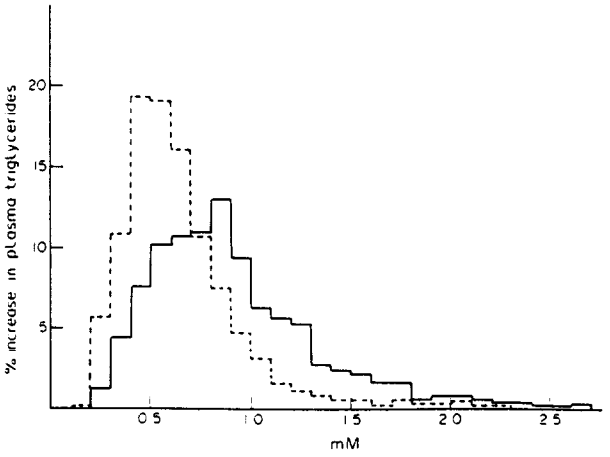

Figure 6
Effect of oral contraceptive treatment on plasma triglyceride levels in women in the 20–40 year age group and less than 15% overweight.

Evidence of induction or inhibition effects. Among the enzymes responsible for transforming drugs, those which require closest monitoring are those associated with the endoplasmic reticulum and whose activity could be reduced or, perhaps more importantly, increased by certain drugs or xenobiotics. Although the direct measurement of such enzymes is sometimes difficult to perform in man, indirect methods are usually available, based either on the assay of endogenous or exogenous metabolites in plasma or urine, or on the measurement of plasma macromolecules or circulating blood cells. Such indirect methods include: antipyrine half-life (hepatic function); $^{14}CO_2$ liberation after administration of labelled aminopyrine (hepatic demethylation); measurement of endogenous metabolite or substrate concentrations; measurement of plasma or urinary drug metabolites; measurement of circulating blood cell enzymes; and measurement of proteins and specific plasma enzymes. An example of the latter method has been discussed above, with respect to GGT.

Early detection of toxic effects. Since drugs essentially affect hepatic function, tests for liver enzymes and bilirubin are amongst those most frequently showing interference. Furthermore, potentially nephrotoxic products can be detected by the measurement of urinary enzyme excretion. *N*-Acetyl-β-D-glucosaminidase has recently been proposed as a sensitive test for nephrotoxicity, with the advantage that it is more specific than other urinary proteins. Useful information on drug toxicity can also be provided by erythrocyte constituents. For example, the initial stages of digitalis intoxication can be detected by the reduction of erythrocyte potassium and the concomitant increase in sodium concentration.

Definition of genetic constitution. The determination of acetylator phenotypes is the most well known example of the use of laboratory tests in this domain. Evans *et al.* [13] have demonstrated that the distribution of plasma isoniazid concentrations in European populations shows a bimodal frequency, with individuals being classed as fast or slow acetylators. If a patient's acetylator phenotype is known, then antitubercular therapy for example, can be adjusted accordingly. In the authors' laboratory an epidemiological

study is currently being conducted on pseudocholinesterase, another well-documented, genetically-important plasma enzyme, which determines a patient's response to suxamethonium-induced anaesthesia [14].

Therapeutic trials. For laboratory tests to be effective, biological variables and analytical parameters such as precision, accuracy and specificity must be carefully defined [11, 12, 15]. This is of particular importance when clinical laboratory tests are used in the course of clinical trials. Some countries such as e.g. West Germany have already recommended the application of a standard analytical programme during animal studies [16, 17].

Database for drug effects on clinical laboratory tests. The Centre for Preventive Medicine performs about 70 000 health examinations per year in eastern France. Since 1971 a questionnaire has been developed to establish the drug treatment being undertaken. The person responsible for taking the blood sample completes one of two questionnaires, depending on the response to two initial questions:

(i) "Do you take the pill [steroidal contraceptive] now, or have you taken it within the last 6 months?"

(ii) "Do you take any medication now, or have you taken any within the last 6 months?"

If the patient replies positively to either of these questions, the questionnaire summarized in Table 1 (for long term treatment) or in Table 2 (for drugs taken only within the preceding 2 days) is completed. The information gathered is coded by pharmacists and then stored on the 'drug file' of the Centre's computer system.

Drugs are classified in 49 groups, the first 20 of which are listed in Table 3. Analysis of the drug file for 1978 revealed that of 37 000 subjects, 9225 (approximately 30%) were receiving some form of drug treatment. Of these, 2849 were male and 6376 female. Table 4 shows the distribution of drug treatment, both for the total population and by sex; long- and short-term therapies are not compared. Some patients received multiple drug therapy and are therefore included more than once.

Table 1
Information requested in long-term treatment questionnaire

Patient identification
Proprietary name(s) of drug(s) taken
Dosage form(s)
Start of treatment(s)
Number of doses per day
Number of days per month
Present treatment
Patient compliance
Has treatment been interrupted? If so, how long ago (0–30 days)?

Table 2
Information requested in short-term treatment questionnaire

Patient identification
Proprietary name(s) of drug(s) taken
Dosage form(s)
Number of doses taken within the last 2 days
Person responsible for initiating treatment: physician, pharmacist, dentist, or patient

Table 3
The first 20 drug classes of the computerized drug file

01 Tranquillizers	11 Vasodilators
02 Neuroleptic agents	12 Hypoglycaemic agents
03 Antidepressant drugs	13 Antihaemorrhagic agents
04 Hypnotics	14 Hypolipaemic agents
05 Sedatives	15 Anticoagulants
06 Psychotonics and vitamins	16 Antiepileptic agents
07 Antianginal drugs	17 Oral contraceptives
08 Antihypertensive agents	18 Antitubercular drugs
09 Antiarrhythmic drugs	19 Antigout drugs
10 Cardiotonics	20 Anorexics

Table 4
Distribution of drug treatment in rank order for 1978 at the Centre for Preventive Medicine, Nancy

	Total population		Males		Females	
	No.	%*	No.	%*	No.	%*
Oral contraceptives	2631	28.5	0	0	2631	41.2
Tranquillizers	1241	13.4	389	13.6	852	13.4
Analgesics	875	9.5	374	13.1	501	7.9
Vasodilators	745	8.1	270	5.5	475	7.4
Gastrointestinal therapy	601	6.5	307	10.8	294	4.6
Vasculotropic agents	600	6.4	114	4.0	486	7.6
Psychotonics	525	5.7	231	8.1	294	4.6
Antiasthmatic agents	494	5.3	283	9.9	211	3.3
Antianginal agents	464	5.0	280	9.8	184	2.9
Antihypertensive agents	446	4.8	185	6.5	261	4.1
Antiinflammatory agents	439	4.8	135	4.7	304	4.8
Antibiotics	387	4.2	178	6.2	209	3.3
Hepatic therapy	309	3.3	87	3.0	222	3.5
Hypnotic agents	287	3.1	91	3.2	196	3.1
Hypolipaemic agents	277	3.0	163	5.7	114	1.8
Diuretics	264	2.9	92	3.2	172	2.7
Antiarhythmic agents	253	2.7	110	3.9	143	2.2
Hypoglycaemic agents	235	2.5	149	5.2	86	1.3
Sedatives	194	2.1	54	1.9	140	2.2
Antidepressants	168	1.8	58	2.0	110	1.7

* Since subjects take more than one drug, they are included several times; the percentage figures therefore exceed 100%.

The most frequently taken drugs were the oral contraceptives, which were taken by 23% of the adult population (41.8% of women were taking this type of drug). These were followed by tranquillizers (13.4%), analgesics (9.5%) and vasodilators (8.1%).

At the Centre for Preventive Medicine a database is being compiled for drug effects on clinical laboratory tests. It comprises information retrieved from patient files, particularly where a single drug is being taken. Another important section of the database concerns the retrieval of information on drug effects from the published literature. Particular emphasis is placed on those tests performed in the authors' laboratory. Details selected from relevant publications are listed in Table 5. The data compiled in this way can be used to study correlations between long-term drug therapy and any detectable modifications in biological components.

The IFCC Expert Panel on 'Drug effects in clinical chemistry' has recognized the

Table 5
Data sought from the literature on drug effects

Identification number	Subjects: man or animal
Bibliographical reference	Number of subjects
Laboratory test	Fed/fasting
Analytical method(s)	State of health
Pharmaceutical name of drug	Age, sex, weight, diet
Proprietary name(s) (if supplied)	Details of control subjects
Drug dose and blood concentration	Animal studies: details of species
Route of administration	Statistical analysis of results
Duration of treatment	Summary of results
Associated treatment	Explanation for the drug effect
In vivo or *in vitro* study	Number of references cited
Biological fluid(s) examined	

necessity for computer storage of data on such drug effects. A document to be published describes the type of information required, most important of which is well-documented quantitative data (N. Tryding, in preparation). Databases on drug effects should be readily accessible to clinicians, clinical chemists, pharmacists, clinical pharmacologists and to other health professionals.

Conclusion

Clinical laboratory tests are useful for following the effects, desirable or otherwise, of drugs or xenobiotics. Because these effects are generally subtle, all other variations must be rigorously controlled and documented [15]. Wherever possible, clinical laboratory scientists should perform tests only on patients whose medication is known. Although this represents the ideal situation, it is an essential condition for the improvement of test interpretation.

Drug effects can result in false diagnoses if the clinician is unaware of interference produced by a drug, although it must be remembered that laboratory tests are but one part of the overall clinical picture. In order that the correct interpretation can be placed on clinical laboratory results, a list of interfering drugs should be drawn up for each method of analysis for each biological parameter. It is equally important to establish which drugs do not interfere.

Further cooperation between pharmaceutical companies and clinical laboratory scientists would contribute enormously towards eliminating the problem of drug effects on clinical tests. It would, for example, be a major step forward if the data sheet for each new drug gave at least minimal details of the principal analytical interferences.

It should not be overlooked, however, that many drug effects on clinical laboratory tests can be useful, particularly for assessing therapy. The subject of drug effects has attracted particular attention in recent years. There is, however, a great need for education and cooperation amongst the health professionals involved, as well as a need for patient education.

Health examination centres are ideally situated for collecting information on drugs, defining the particular drugs at risk and using the accumulated data for information and education. In this context, clinical scientists are well placed to collaborate both with physicians, in assessing the relevance of drug effects on clinical tests, and with pharmaceutical analysts, in developing new strategies for the sensitive and selective analysis of drugs, metabolites and endogenous constituents.

References

[1] G. Siest, M.M. Galteau and D. Notter (Comité Scientifique de la Société Française de Biologie Clinique, Section de Physiopathologie, Commission: Effets des médicaments sur les examens de laboratoire), *Ann. Biol. Clin.* **39**, 91–98 (1981).
[2] A. Galli, R. Galimany and M.M. Galteau (Comité Scientifique de la Société Française de Biologie Clinique, Section de Physiopathologie, Commission: Effets des médicaments sur les examens de laboratoire), *Inform. Sci. Biol.* **3**, 107–112 (1981).
[3] M.M. Galteau *et al.*, in *Topics in Clinical Chemistry*, Walter de Gruyter, Berlin (in press).
[4] D.S. Young, K.C. Pestaner and V. Gibberman, *Clin. Chem.* **21**, 1D–432D (1975).
[5] G. Siest *et al.*, *J. Clin. Chem. Clin. Biochem.* **16**, 103–110 (1978).
[6] B.E. Statland, H. Bokelund and P. Winkel, *Clin. Chem.* **20**, 1513–1519 (1974).
[7] D.J. Greenblatt, R.I. Shader and J. Kochweser, *J. Clin. Pharmacol.* **16**, 118–121 (1976).
[8] *Drug Effects in Clinical Chemistry*, Apoteksbolaget, Stockholm (1981).
[9] F. Schiele *et al.*, in *Drug Measurement and Drug Effects in Laboratory Health Science*, p. 185 (G. Siest and D.S. Young, Eds), Karger, Basel (1980).
[10] M.M. Galteau *et al.*, in *C.R. 2ème Symposium de Biologie Clinique Carcinologique*, p. 281 (C. Bohuon and J.C. Jardillier, Eds), Reims (1978).
[11] P. Métais *et al.*, in *Biochimie Clinique–Biochimie Métabolique*, (P. Métais, Ed.), p. 25. Villeurbanne, Simep, France (1980).
[12] G. Siest *et al.*, *Ann. Biol. Clin.* **37**, 229–239 (1979).
[13] D.A.P. Evans, K.A. Manley and V.A. McKusick, *Br. Med. J.* **2**, 485–491 (1960).
[14] M. Whittaker, *Anaesthesia* **35**, 174–197 (1980).
[15] G. Siest, J. Henny and F. Schiele, Eds. *Interprétation des examens de laboratoire*, Karger, Basel (1981).
[16] J. Breuer *et al.*, *J. Clin. Chem. Clin. Biochem.* **14**, 161–164 (1976).
[17] J. Breuer, *Dtsch. Ges. Klin. Chemie Mitteilungen* **5**, 146–147 (1977).

[*First received for review 4 October 1982; revised manuscript received 13 May 1983*]