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PHARMACOLOGICAL AND TOXICOLOGICAL ASPECTS OF QUALITY CONTROL

by

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The problems of quality control of pharmaceutical preparations are manifold. The ultimate aim of the application of such control methods is to ensure the arrival of the therapeutic agent to the patient in the most assimable form with the highest potency and least possible toxicity. This aim can be achieved in actual practice only after detailed studying and follow up over a long period, a procedure which has been possible to follow only in a few numbers of therapeutic agents of wide-spread use and major importance.

The first problem that faces the responsible bodies in this field is the availability of the required data concerning the ideal specifications of the active principles and different ingredients as well as those related to the conditions of processing, manufacturing, packaging and storage under which the original properties of these active principles and ingredients can be retained until their ultimate administration to the patient.

Acquiring such a mass of information, assessing their validity and sorting out their relevant significance is the function of different official and non-official bodies in each country.

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From the experience of recent years it is obvious that for the present no single country in the world has mobilized the required resources needed to face the gigantic problems created by the continuous flood of newly introduced therapeutic agents or by the deficiency in our concepts and knowledge of the already established ones.

While it is only fair and practical to expect the quality control activities to attempt to cover only the area lighted by the generally accepted and established data yet in the continuously shifting and developing scientific fields affecting the drug industry, quality control bodies whether connected with the manufacturing organizations or with the states structures have often to use their own judgement and experience to supplement or alter the already established criteria to-date.

The problems of the quality control in industry are different in this aspect from those facing state quality control. Because of better facilities, narrower scope of responsibility and often richer experience and a much higher degree of malleability in both their approach and position they are able to take the lead in supplying most of the information ultimately to be integrated in the official regulations.

The state official control bodies on the other hand with their wider scope of responsibility, often with limited resources and the far reaching repercussion of their decisions have to be more conservative in both their approach and stand.

A new complication which has arisen in recent years affecting fundamentally relationship between these two bodies in certain type of states needs some elucidation. In states where the drug industry passes over to public ownership, whether it is in its importation section or in the manufacturing section as well, the role of the state control bodies, as well as that of the factory control laboratories undergo an important development.

From the point of view of the central quality control organizations, a new function is added to the previous accepted role; that is the neccesity to help the public ly owned industry solve its problems and achieve the required level of efficiency and accuracy and while the main function of

ultimate control remains of primary importance, a new role of advisor, helper and co-worker would often affect both the approach and the stand of these central bodies.

A parallel shift is often noted in both the approach and the stand of the factory quality control people with the more manifest readiness to arrive at a common stand and an understandable tendency to sharing responsibilities and co-ordinating policies.

It is to be noted however, that in our experience the difference between the two groups in this type of state from the point of view of role, facilities as well as experience is much narrower than it is usually.

In addition the newly developing industries in these states with the conditions imposed by the centrally planned economy faces very special problems which certainly affect very much the whole concerned organizations.

The special problems created by rapidly increasing population and the parallel vastly increasing requirements of health services, almost previously non-existent, and more often with very limited financial resources and existing manufacturing facilities and experience; all this necessitates the utmost efficiency in the use of the available resources.

Bulk purchase of raw materials and finished pharmaceutical specialities under conditions of restrictions on foreign currency expenditure, leads to the necessity of detailed scrutiny in the choice of products and raw materials to be selected. With the astonishing variations in quality and prices and confusing claims of various suppliers, such decisions cannot in any honesty be taken except with continuous help of well experienced laboratory services.

In fact, the meaning of quality control in this type of service tends to advance beyond the usually accepted limits. It is not enough any more to verify that a certain item conforms to the officially required specifications, quite often a comparative quality study of similar preparations is needed which does not stop at the mere comparison of the officially stated limits but proceeds to the evaluation of the significance of these differences and their ultimate effect on both potency and toxicity under the prevailing local conditions of importation manufacturing, storage, distribution and administration.

It is within the scope of this new shift in the meaning of quality control in developing countries that we have found a need for extending the scope of our activities. While employing the most advanced techniques in the analytical fields, e.g. ultraviolet and infrared spectrophotometry, polarography, gas chromatography etc, as routine techniques and still more advanced techniques for specific purposes, we still find that biological studies are often needed to give a proper assessment of the items under investigation.

I would like to give you some examples of such situations where pharmacological or toxicological studies were needed to give meaning to our findings by the usual analytical, both physical and chemical, methods.

Example I

Antimony potassium tartarate is still the drug of choice in treatment of Bilharziasis in Egypt, and in other infested areas, though it has been in therapeutic usage since the early years of this century.

Antimony potassium tartarate, however, is obtained by the interaction of Antimony trioxide and potassium acid tartarate. These compounds are usually contaminated with impurities of other heavy metals, mainly lead and arsenic. The role played by these impurities and others that may be present as well as by other chemical differences that may be present in different batches of tartar emetic prepared from different sources of raw materials and by different processes has not been investigated

While it has been recognised that such impurities may play an important role, and that the method of preparation and presentation may also be of importance, scientific evidence to support these views was still lacking to an extent reflected in the limits allowed by different pharmacopoeias.

Difficulties in importing tartar emetic with very low impurities of lead and arsenic from the biggest known international suppliers led to complete paralysis of our antibilharzial campaign in the early sixties. Not only did we have to ensure a thorough quality control of the multitude of products that converged from all over the world but a proper assessment that goes beyond the arbitrarily fixed official limits had to be carried out

and a rational and practical advice given to the authorities, then we carried out three additional studies after exhausting the official tests.

1. A comparative study to investigate the relationship between impurities and toxicity.
2. An investigation of stability in relation to storage and container. This investigation led to the actual change of method of production and presentation.

Effect of Purification by Recrystalisation on Toxicity

Discussion

Toxicity of tartar emetic obviously is affected by the presence of impurities of heavy metal salts and possibly other compounds. The view held by Abdallah et al. (1962) that such impurities could be taken as an indicator of the degree of care in manufacturing processes, is confirmed by our experience. The raw materials available in the international market vary in their degree of purity to an amazing extent. Additional factors affecting the degree of purity can be easily incurred during processing and presentation.

The toxicity of individual batches, however, did not always follow a constant pattern. It became obvious during our investigation of repeated lots of tartar emetic, some imported from the best known foreign manufacturers and some processed locally in one of our factories, that while the concentration of lead and arsenic present seemed to part, it could not explain totally the shifts in toxicity.

In chemically impure lots removal of arsenic and lead did not always lower toxicity to the expected level neither did additions of these salts to highly purified tartar emetic always raise toxicity to the expected level.

Further purification by recrystalisation, whether resulting into further reduction of heavy metal impurities or not, seemed to reduce the toxicity of the drug.

The L.D. 50 for tartar emetic before chemical purification, agrees fairly accurately with that reported by Stecher et al. (1960). Our estimation is 60 mgm/kgm body weight for mice receiving the drug intraperitoneally that of Stecher et al. is 50 mgm/kgm. The curve for which it is calculated

Recrystalisation caused further decrease in toxicity (L.D.50 much nigher than 83.3 mgm/kgm, the highest level tested). It is of interest to notice that the mother liquor after recrystalisation retains a high degree of toxicity Table (1), Fig. (1)

Fig. (1)

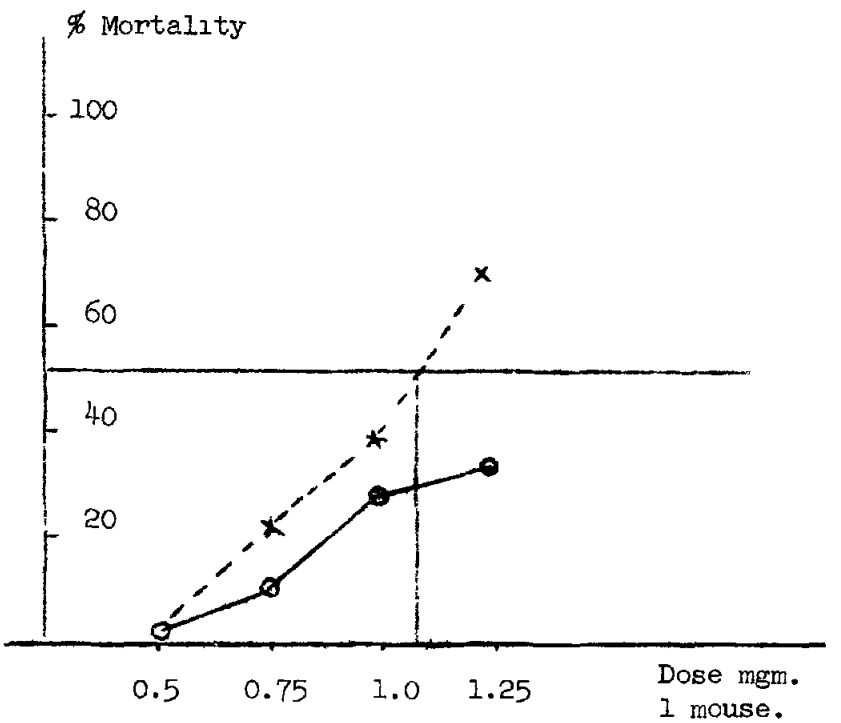


TABLE I

Number of Experiment	Tartar Emetic	Chemical Analysis		No.of mice per group	% Mortality				Estimated LD 50
		Arsenic	Lead		0.5mgm	0.75	1.0	1.25	
I	Chemically purified	100	45	65	3.0	18.5	51.5	73.5	60.- mgm/kgm
	Chemically purified A	5	3	100	2.0	20	45	64	70.6 mgm/kgm
II	Chemically purified B	4	4	40	2.5	22.5	37.5	67.5	73.3 mgm/kgm
	B After recryst.	3	4	40	2.5	10	27.5	32.5	above 83.3 mgm/kgm

Antibilharzial Activity of Antimonyl Potassium Tartarate as Influenced by the Colour of the Glass Container on Storage

Parallel tests of antibilharzial activity of two batches of tartar emetic solution, one stored in brown (dark) glass ampoules for 29 months and the other in white (colourless) glass ampoules for 24 months, carried out against experimental schistosoma mansoni infection in mice, revealed that both batches were apparantly equally potent insofar as the stools of treated animals were rendered negative for the presence of bilharzia ova on the 10th day after the commencement of treatment. The worm load collected by perfusion of liver and portocaval system of infected mice at the end of T.E. treatment with any of the two solutions showed considerable reduction in the total number of worms of which 1/3 were virtually dead while those still alive were stunted and sluggish in movement. However, judging by the results of stools examination and inspection of worm load collected by perfusion of the treated animals after eight weeks follow-up period, T.E. solution stored in dark glass ampoules proved to be far superior in anti-bilharzial activity than the batch of the same organic antimonial kept in colourless glass containers. The relapse rate among treated mice at that time as evidenced by reappearance of ova in stools was much less frequent in the animal group receiving T.E. stored in dark ampoules than in those receiving the batch of the drug in colourless ampoules, the rate in the form instance amounting to 25% as compared to 50% in the latter. Likewise, the worm load which was still low consisting of living worms with peculiar reduction of females and absence of coupling forms in mice treated with T.E. solution stored in dark ampoules contrasted with a relatively higher number of viable worms with reappearance of coupling due to the presence of females constituting almost 1/5 of the total number recorded in the group of animals injected with T.E. in colourless ampoules. Tables (2), (3) and (4) Fig., (2) and (3).

COMPARISON BETWEEN THE POTENCY OF STORED AMPOULES OF T.E. AND FRESHLY PREPARED SOLUTION (Dose 25mg/kg/day for 6 doses I.P.)

	1 Year Stored Amp	Fresh Solut	Control	15 mon stored Amp	Fresh Solut	Control	20month stored Amp	Fresh Solut.	Control	22month stored Amp	Fresh Solut.	Control
No. of living worms in each group	1	2	300	-	-	265	2	1	280	2	3	270
% of living worms as compared to control	0 3	0.66		~4	0		0 7	~0 3		~0 8	1 1	

TABLE 3

ANTIBILHARZIAL ACTIVITY OF ANTIMONYL POTASSIUM TARTARATE (TARTAR EMETIC CID) TESTED AGAINST EXPERIMENTAL SCHISTOSOMA MANSONI IN MICE RECEIVING I.P. DAILY DOSES mg/kg BODY WEIGHT FOR SIX SUCCESIVE DAYS AS INFLUENCED BY THE COLOUR OF THE GLASS CONTAINER ON STORAGE

Animal Group		Stools examination of infected mice for ova performed 2 months after exposure to cercariae	Worm load collected by perfusion of liver and portocaval system of infected mice at autopsy after treatment or follow-up period					Remarks
			Males	Females	Coupling	Total Alive	Total Dead	
A) Control Mice		+ve	67	70	195	332	-	All worms were living actively motile and showed high incidence of coupling
B) Treated Mice injected with T.E solution batch No.318 stored in brown dark glass ampoules for 29 months Date of Manufacture - May 1964	Result of treatment	100% Cure - Stools becoming -ve on 10th day after commencement of T E course	40	-	--	27	13	Note considerable decrease in number of worm with no coupling
	Follow-up period of 8 weeks after commencement of T.E course for relapse	25% Relapse	18	18	-	36	--	A significant proportion of the worms viz 1/3 of the total number were dead at the termination of treatment while those still alive were stunted and sluggish in movement 25% relapse showing +ve stools 8 weeks after commence of treatment
C) Treated Mice injected with T E. solution batch No 314 stored in white (colourless) glass ampoules for 24 months Date of Manufacture- September 1964	Result of treatment	100% Cure - Stools becoming -ve on 10th day after commencement of T E. course	28	10	--	24	14	Marked reduction in number of worms and absence of coupling after termination of T E. course
	Follow-up period of 8 weeks after commencement of T E course for relapse	50% Relapse	92	12	24	128	--	Higher incidence of relapses (50%) with reappearance of coupling forms of the worms at the end of the follow-up period

TABLE .

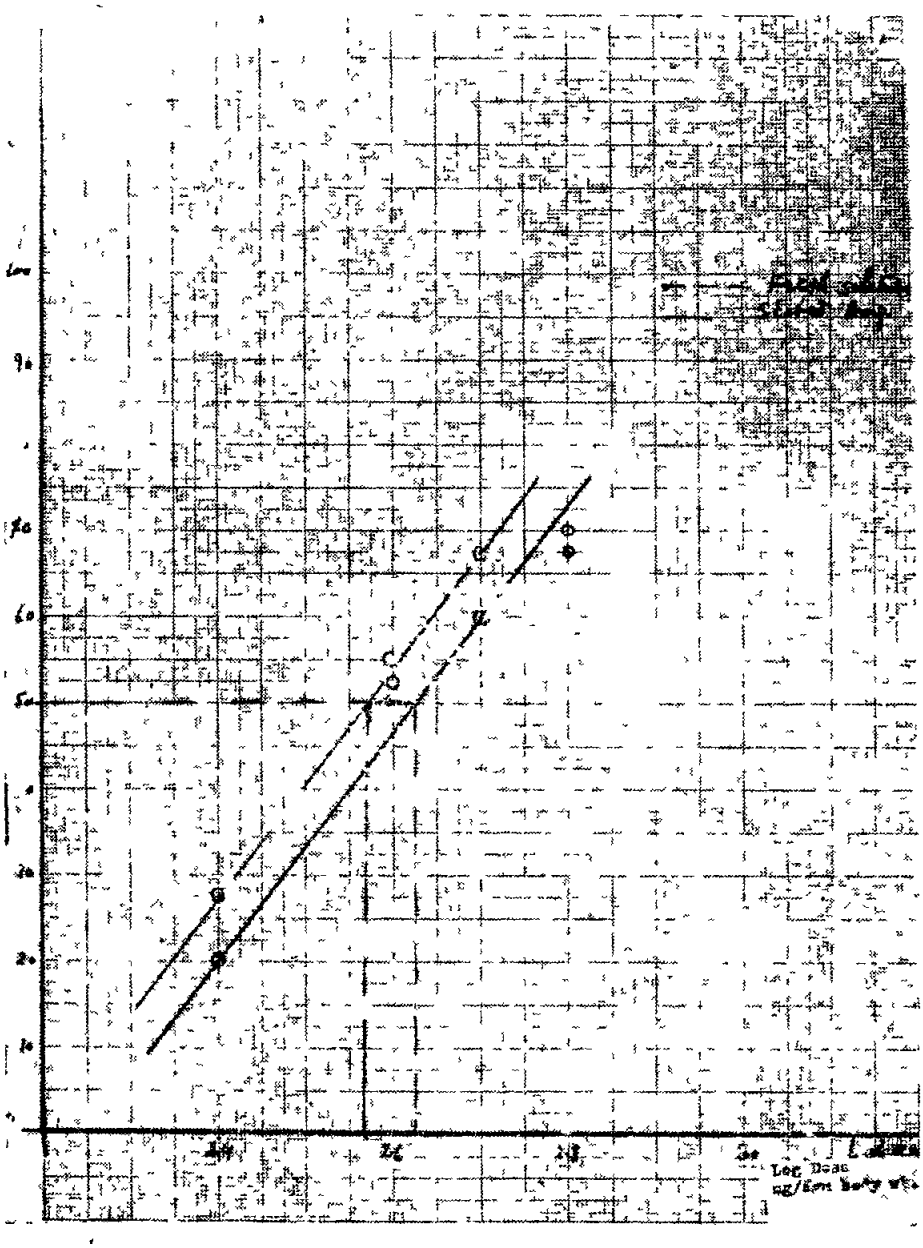
DATA COMPILED FROM THE RESULTS OF THE SPEARMAN-KARBER METHOD SHOWING THE LEVEL OF ACUTE TOXICITY OF TARTAR EMETIC (TE · POTASSIUM ANTIMONYL TARTRATE, CID) FOLLOWING ITS INTRAPERITONEAL (IP) INJECTION IN MICE AS INFLUENCED BY THE COLOUR OF THE GLASS CONTAINER AND THE DURATION OF ITS STORAGE

No of animals in each of the control and injected groups « 6 mice »

Test doses of tartar emetic (TE) per kg body weight expressed		No of deaths occuring within 21 hours among groups of animals receiving IP test doses of the following batches of TE					
		(1) Control group injected with normal saline solution having the same pH as TE solution	(2) Freshly prepared 6% TE solution supplied in white (colourless) glass ampoules of 2 ml capacity Batch No 334 (Standard) Date of Manufacture Feb 1966	(3) 6% TE solution stored for 22 months in brown (dark) glass ampoules of 2 ml capacity Batch No 319 Date of Manufacture May 1964	(4) 3% TE solution stored for 18 months in white (colourless glass ampoules of 10 ml capacity Batch No 314 Date of Manufacture Sept 1964	(5) 6% TE solution stored for 29 months in white (colourless) glass ampoules of 2 ml capacity Date of Manufacture Oct 1963	
(1) logarithmic scale	In mg					(a) Batch No 311	(b) Batch No 315
1·1	12·5	0/6	0/6	0/6	0/6	0/6	0/6
1·2	16·0	0/6	0/6	0/6	0/6	0/6	1/6
1·3	20·0	0/6	0/6	0/6	2/6	1/6	1/6
1·4	25·0	0/6	0/6	0/6	0/6	1/6	0/6
1·5	32·0	0/6	0/6	1/6	1/6	1/6	2/6
1·6	40·0	0/6	2/6	2/6	1/6	4/6	4/6
1·7	50·0	0/6	3/6	3/6	6/6	6/6	5/6
1·8	63·0	0/6	6/6	6/6	6/6	5/6	6/6
1·9	80·0	0/6	6/6	6/6	6/6	6/6	6/6
2·0	100·0	0/6	6/6	6/6	6/6	6/6	6/6
Values for Intraperitoneal Median Lethal Dose (IP LD_{50}) of TE calculated from the formula $\text{Log } LD_{50} = X_k + k\left(d - \frac{d_1}{n}\right)$ expressed in mg/kg body weight of mice			46·42	44·67	34·11	30·45	32·86
5% Fiducial Limits for IP LD_{50} Values i — Lower Limits ii — Higher Limits			43·24 49·72	41·87 48·14	29·26 39·84	27·88 33·21	30·11 35·57
Relative Toxicity of various test batches of TE as judged by ratios obtained by division of the IP LD_{50} of the freshly prepared sample vi Batch No 334 by the corresponding median lethal value of			1·00	1·04	1·36	1·53	1·41

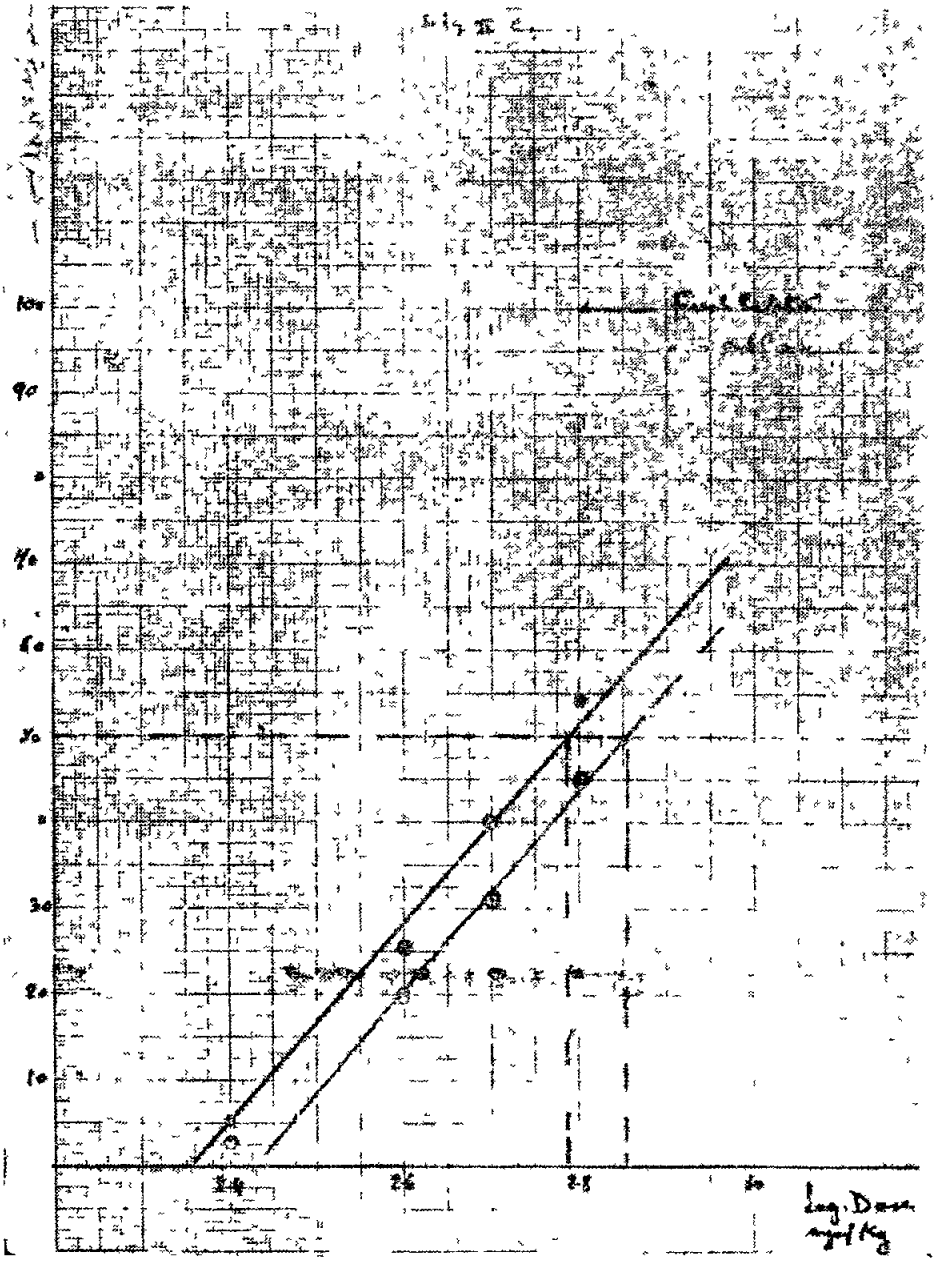
3rd Experiment after 18 months of storage

Fig 2



b Experiment after 24 months of storage

Fig 2



Acute toxicity determination of various stored batches of T.E. solution performed in parallel experiments using a freshly prepared sample of the antimonial as a reference standard indicated that storage of T.E. solution in brown (dark) glass ampoules for 22 months did not lead to any significant increase in its toxicity relative to that of the freshly prepared sample. On the other hand, storage in white (colourless) glass ampoules for periods of 18 and 22 months markedly enhanced the toxicity of T.E. solution by values amounting to 1/3 and 1/2 the level of toxicity of the reference standard. Only on the results of these investigations could a decision be taken regarding the conflicting claims and justifications in this important problem.

Example II

Broad spectrum antibiotics represent an important sector of our concern both therapeutically and financially.

In no other sector of the drug industry has the flood of supplies with variations in specifications, claims and prices confused the planner and the user. The decision is often critical and handicapped with multitude of pitfalls. Considering the vital importance to the user and the expenditure of the community such a decision may often mean safeguarding the safety of hundreds of thousands of seriously ill patients with the limited resources in certain countries, the difference in prices may also mean the question of the availability of the antibiotic to the whole community or only certain strata. We have been faced with the neccesity of a detailed study of the quality of multitude of preparations who fulfilled all the official specifications both chemically and microbiologically but still had such vast differences in fame, popularity, presentation and most important price, to an unbelievable degree.

We give parts of our investigations in two of these cases.

Comparative studies of chloramphenicol activity, fate and absorption in different suspensions

Numerous esters of chloramphenicol have been prepared. The palmitate ester has become popular for oral paediatric administration because it lacks the bitterness of free chloramphenicol.

Larkin (1951) described such a compound in which one hydroxyl group of the propandiol side-chain is esterified by palmitic acid to form chloramphenicol palmitate.

Chloramphenicol palmitate which is a white crystalline substance is virtually devoid of any antibacterial activity, but it is readily hydrolised in the duodenum with liberation of free chloramphenicol which is available for absorption from the upper intestine.

It has been claimed that absorption of chloramphenicol palmitate in suspension form is related to the particle size as well as to its form. Small sized and amorphous forms were claimed to be the best as regards the rate of absorption as they are more amenable to enzymatic hydrolysis than larger crystalline forms.

The antimicrobial activity of the administered antibiotic in the biological fluids is the only reliable criteria for evaluating the rate of absorption of the antibiotic under consideration.

This work has been carried out to verify such claims and to decide on the most suitable preparations.

Materials and method

Chloramphenicol suspension was administered by mouth to dogs after administering a dose of 50 mg/1 kg. body weight. The serum antibiotic level was assayed microbiologically. Samples of blood were taken from the animals 2 hours, 4 hours and 6 hours, after administration. As a control blood samples were taken from the animals 2 hours before administering Chloramphenicol to serve as a blank for the assay.

Samples of Chloramphenicol suspension produced by different suppliers were tested with the object of evaluating the rate of absorption in the animals.

Each suspension was given to three dogs, and, therefore, four groups of dogs each consisting of three were used for the four different preparations. Table (5) and (6).

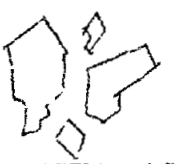
Results of Microbiological Estimations of
Antibacterial Actions of Serum of Orally Treated Dogs
With Chloramphenicol Suspensions

Suspension Dog No.	Mean Inhibition Zones of 5 Inoculations		
	Serum after 2 hours	Serum after 4 hours	Serum after 6 hours
1	30	Slight	0
2	28	Slight	0
3	30	Slight	0
4	31.5	Very Slight	0
5	31	Very slight	0
6	29.5	Zero	0
Total mean.	30	--	-
S.D.	+ a5	--	-
1	30	Very slight	0
2	28	Very slight	0
3	28	Very slight	0
4	27.5	Zero	0
5	26.5	Zero	0
6	28	Zero	0
Total mean.	28	--	-
S.D.	+ 0.567	--	-
1	28.5	Slight	0
2	28	Slight	0
3	27	Very slight	0
4	27	Very slight	0
5	29.5	Very slight	0
Total mean.	28	--	-
S.D.	0.4	--	-
1	27	Zero	0
2	27.5	Zero	0
3	26.5	Very slight	0
4	27	Zero	0
5	28.5	Zero	0
6	25.5	Slight	0
Total mean.	27	--	-
S.D.	0.43	--	-

TABLE 5

Table 6

Chloramphenicol Activity in 24 hrs Urine of Rabbits

Suspension	Rabbit No.	mean inhibition zones 85 inoculation	crystal size and shape
I	1 2 1 & 2	13 13 13	
	Total mean S D	13 0	
2	1 2 1 & 2	10 5 11 5 11	
	Total mean S D	11 ± 0 201	
3	1 2 1 & 2	11 11 11	
	Total mean S D	11 0	
4	1 2 1 & 2	9 5 10 10	
	Total mean S D	10 0 143	

Comparative Clinical In Vivo Evaluation of the Rates of Absorption and Excretion of Different Commercial Brands of Oxytetracycline Hydrochloride

Summary

Comparative clinical in vivo evaluation of the rate and extent of gastro intestinal absorption and of the urinary excretion levels of three imported commercial brands of oxytetracycline hydrochloride has been conducted on randomized groups of normal adult male volunteer subjects receiving single oral doses of one gram each.

The serum concentrations of the antibiotic as determined by microbiological assay in blood samples withdrawn at intervals of 2,4 and 5 hours after drug ingestion were taken as criteria of the rapidity and extent of alimentary absorption. The total 5 hour urinary excretion level of each brand of the antibiotic served as basis for comparison of the rates and degree of their elimination by the Kidney. Fig. (4) and (5), (See page 18).

Conclusion

These results allowed us to pacify our growing uncertainty about certain preparations which while fully confirming official and international specifications often exhibited too wide a difference in price and fame.

Often a follow-up with clinical trial was resorted to in vital questions as did actually happen with tartar emetic.

Similar problems regarding analgesic preparations, as well as hypoglycaemic agents and anticoagulants could not be resolved by all the known analytical procedures and techniques. A supplementary biological or toxicological study was necessary before the final decisions regarding these agents could be taken.

While such an extension of the role of the quality control bodies should not be allowed to overwhelm their limited facilities.

We believe that in developing countries the expenditure and effort given to such fuller investigations often allow for better understanding and co-operation from the concerned people in the industry as well as the medical services. All and above there is often a greater saving of resources and firmer insurance of their proper utilization.

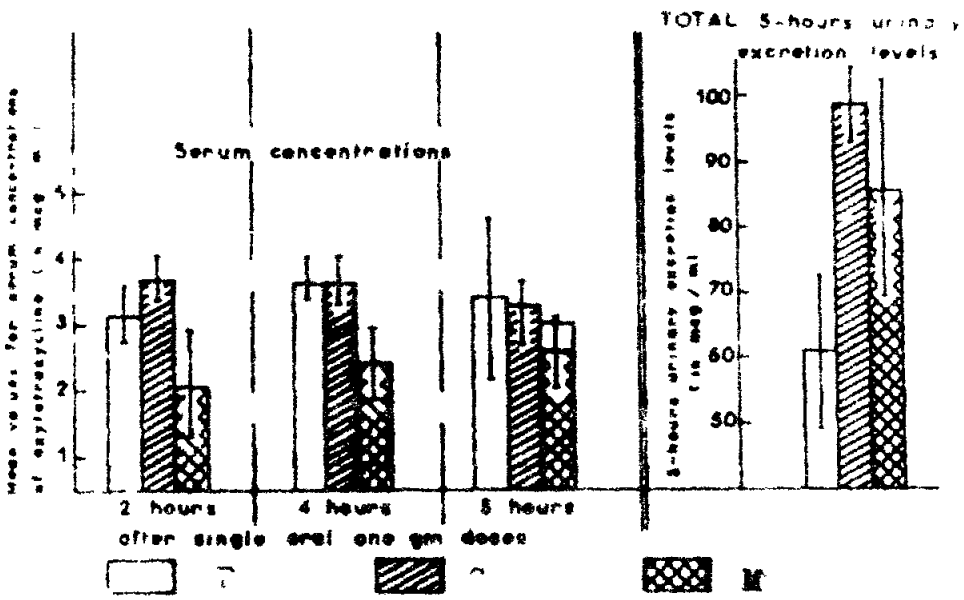


Fig (7) SERUM CONCENTRATIONS OF THREE BRANDS OF OXY-TETRACYCLINE AND THEIR 5 HOURS URINARY EXCRETION LEVELS AS DETERMINED IN GROUPS OF NORMAL MALE VOLUNTEER SUBJECTS AT SPECIFIED TIME INTERVALS

Range of S D shown as vertical lines on top of the bars

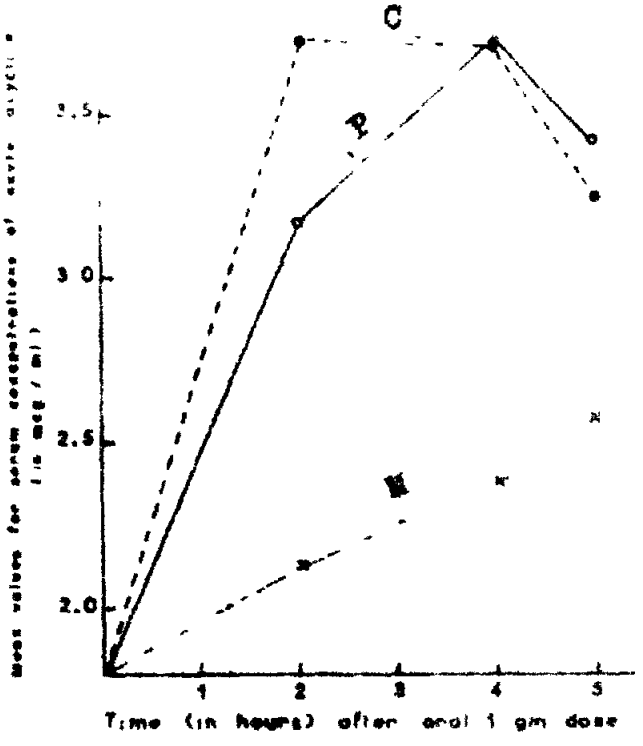


Fig. 5 . SERUM CONCENTRATIONS OF OXYTETRACYCLINE FOLLOWING ORAL ADMINISTRATION OF SINGLE ONE GM DOSES OF THREE DIFFERENT COMMERCIAL BRANDS IN GROUPS OF NORMAL MALE HUMAN VOLUNTEER SUBJECTS