**REPORT ON THE TESTING FOR ARTIFICIAL
STIMULANTS IN URINE SAMPLES FROM
FOOTBALL PLAYERS IN THE WORLD CHAM-
PIONSHIP**

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by

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The tests were carried out in accordance with the Anti-Doping Regulations laid down by the Medical Sub-Committee of F. I. F. A., in collaboration with the Football Association and the Department of Pharmacy, Chelsea College of Science and Technology.

The protocols outlined below were evolved and applied from experience obtained in 'anti-dope' testing carried out during the Tour of Britain (1965 and 1966) cycle races.

SAMPLING

Sampling Scheme

Random sampling was undertaken at all matches.

Table 1

Area	Grounds	No. matches	No. players sampled	No. players unable to give samples	No. controls sampled
1	Wembley and White City	10	40	-	7
2	Sheffield and Birmingham	7	28	-	1
3	Manchester and Liverpool	8	31	1	3
4	Sunderland and Middlesbrough	7	26	2	1
TOTALS		32	125	3	12

Samples from four players were taken at each match (two from each team) except at the Wembley semi-final (6 samples, 3 from each team), the Final (2 samples, 1 from each team made up to 4 samples with two blank controls) and in the cases where a player was unable to urinate. Control samples were included in addition. All samples were 'divided' (see Sampling Procedure).

There was also provision for additional demands for the testing of particular players to be made by the Referee or the F.I.F.A. Commisar at the end of each particular game. Such testing was not found necessary however.

Sampling Officers

In each area two sampling officers, appointed by the laboratory, were responsible for collection of samples at each match. These officers were issued with identity cards to allow ease of access to the grounds. If substitution of an officer at any match became necessary, Central Medical Liaison was informed and the name of the stand-in officer was telexed to the particular ground.

Selection of Players for test

The players for test were selected by ballot before each match, by the Referee (or F.I.F.A. Commisar) and the Area Medical and Sampling Officers. There were slight variations in the method of selection from area to area. The method adopted at Wembley was as follows:

Before each match the identity cards of the players in the two teams were taken to the Referee's room where the Referee selected two from each team at random. This was done in the presence of the Area Medical and Sampling Officers. The Sampling Officer then prepared three letters indicating the names and numbers of the players who would be required at the end of the match. One was addressed to the F.I.F.A. official responsible for marshalling the selected players to the medical room at the end of the match, and the others were addressed to each of the team doctors. These letters were delivered by the Sampling Officer as the teams went onto the field for the second half of the match.

Preparation for Sampling

In order to avoid confusion at the end of the match all preparations were made before it started.

(a) Labelling of bottles: The bottles used (16 fl. oz. oval emulsion

bottles) were obtained directly from the warehouse. Three labels were used on each bottle. A circular label was affixed to the screw cap and a large rectangular label to the side of each bottle. The code number of the particular bottle was written on both of these labels. They also bore the name and address of the Chelsea Department of Pharmacy. The third label, bearing the signature of one of the sampling officers, was also prepared but was not affixed at this stage.

A three part code was employed, consisting of -

- (i) The area number (see Table 1)
- (ii) The date (day) number
- (iii) A letter designating the particular player

For example: 3.19.A represented Liverpool. 19th July, Pereira J.

- (b) Preparation of the record book: Each area was provided with a register. A specimen page is shown in Fig. 1.
- (c) Preparation of sealing fluid: A cellulose acetate solution was employed.

During the match the coded bottles and the register were kept in a sealed box in a safe place.

Sampling Procedure

In addition to the sampling officers, the following persons were required to be present throughout the sampling procedure:

- (i) The Area Medical Officer (also present in his capacity as a F.I.F.A. official)
- (ii) The Doctor or Manager or other suitable representative of the team whose players were involved.

The presence of an interpreter was also essential in some cases.

The sealed box containing the coded bottles and the register was opened at the end of the match in the presence of the Area Medical Officer.

The players were in turn given any one of the coded bottles. Each player was then requested to urinate into the bottle in the presence of the

Area Medical Officer. If the player could not urinate immediately he was requested to remain available until he could do so (see Appendix 1).

The player then returned the sample to the Sampling Officer who, in the presence of the player, poured half of it into a second bottle bearing the same code number.

A label bearing the signature of one of the sampling officers was then affixed across from the cap onto the neck of each bottle. The neck of each bottle was then rotated in the sealing fluid. The fluid quickly dried out to give a firm, transparent layer covering the cap and signature label. It was quite impossible to tamper with this seal without leaving evidence of having done so.

The player was then requested to sign the record book in duplicate, alongside his name and bottle code number to verify that he had witnessed the whole procedure.

Only then was the player allowed to leave the room.

The whole procedure did not normally take more than five minutes.

After sampling was completed, the two sets of duly sealed specimens were placed in separate boxes which were then sealed with a distinctive F.I.F.A. metal seal and the fact of their sealing verified by the sampling officers and the Area Medical Officer (or other F.I.F.A. officials).

The master record of the names of the players tested together with the numbers of their specimens was kept in a safe place by the sampling officers. A letter, addressed to Prof. Andrejevic the President of the Medical Sub-Committee, F.I.F.A. H.Q. marked "Confidential", sealed with sellotape and containing a copy of the master record of the names of the players tested on that particular day together with their bottle code numbers was included with the set of samples for dispatch to the laboratory.

Delivery of Samples

One set of samples was held by the Area Medical Officer and kept available, for further division and analysis, in case of dispute. They were not destroyed until official permission had been received from F.I.F.A. H.Q. These samples were kept in a safe place and refrigerated.

The other set of samples was taken by the sampling officers for personal carriage to the laboratory or by express rail delivery to a London

terminus to await collection by a laboratory representative.

A receipt for dispatch of the samples was obtained from British Railways and was stuck in the appropriate page of the record book.

In all cases samples were received at the laboratory on the morning after the day on which the game in question had been played.

The Sampling Officer in each area was in telephone contact with a member of the laboratory staff after each game to give details of the time of arrival and destination of the train on which he had consigned the samples.

Control Samples

From time to time, urine from volunteers who had taken stimulants was also sent to the laboratory. These samples were coded in the same way as the samples from the players and slipped into a series. Their function was to act as a check on the experimental method and the organisation of the testing.

ANALYSIS

Analysis Protocol

All samples were analysed in the Pharmacy Department at Chelsea College of Science and Technology within six hours of receipt. Analyses were performed under the supervision of Professor A.H. Beckett, D. Sc., Ph.D., F.R.I.C., F.P.S.

As the samples arrived at the laboratory, two members of the staff were required to verify, by signature, that the seal was intact and the box was opened in their presence. The seals on all specimens were similarly examined (see Fig. 2, a copy of one of the laboratory data report sheets which were used).

The sealed letter accompanying each set of samples and addressed to the President of the Medical Sub-Committee, was conveyed immediately to F.I.F.A. H.Q. where it was given to the President in person or to his Secretary.

Analyses were performed in the presence of at least one person other than the analyst. The analysts were not aware of the relationship between the code numbers of the bottles and the names of their owners. The code could only be broken by the appropriate sampling officers and the

President of the Medical Sub-Committee.

F.I.F.A. officials could also be present during the analyses if they so desired

Analytical Methods

Most of the drugs covered by the analytical scheme came under the general heading of 'artificial stimulants'. They would be used for short-term pharmacological conditioning when there is a direct relationship between the time of use of the drugs and the physical effect of the athlete

A list of some of the drugs covered by our normal screening procedure is given in Table 2. The list is not comprehensive but serves to indicate the wide range of compounds for which the analytical scheme is devised.

Nicotine, although a stimulant, was not classified as a prohibited drug since it would be excreted as the result of tobacco smoking.

Caffeine is a similar case since it is present in normal beverages such as tea and coffee.

The analytical procedure consisted of three phases:

Phase 1 Routine screening using a method based on gas-liquid chromatography (G.L.C.). Four gas chromatographs were employed routinely; two with an 'amphetamine column' and two with an 'ephedrine column'. For an account of the techniques see -

1. Beckett A.H. and Rowland M (1965) J. Pharm. Pharmacol 17, 59-60.
2. Beckett A.H. and Wilkinson G.R. (1965) J. Pharm. Pharmacol. 17 104S - 106S.

Phase 2 Confirmatory identification using selective reagents and G.L.C. of the drug derivatives formed with them. (e.g. Amphetamine forms Schiff's bases, with many ketones and aldehydes; acetyl; propionyl and CS₂ derivatives.) Also, thin-layer chromatography (T.L.C.) of drugs and their metabolites.

Phrase 3 Use of preparative G. L. C. and/or T. L. C. (if sufficient drug is present) followed by I. R., O. R. D. (if the compound is optically active) and mass spectroscopy techniques on the separated sample.

(25% of the samples were found to contain nicotine).

Table 2 Some of the Drugs detected by Normal Screening Procedure

Amphetamine (Benzedrine)
Methylamphetamine (Pervitin)
N-Ethylamphetamine (Adiparthrol)
Phentermine (Linyt, Ionamin)
Mephentermine (Wyamine)
Dimethylamphetamine
Diethylamphetamine
Propylhexedrine (Benzedrex)
Cyclopentamine (Clopane)
Nicotine
Ephedrine
Methylephedrine
Norephedrine (Phenylpropanolamine, Propadrine)
Ethylephedrine
Benzphetamine
Chlorphentermine (Lucofen)
Tranylcypromine (Parnate)
Phenmetrazine (Oxazimedrine, Preludin)

Phendimetrazine

Methoxyphenamine (Orthoxine)

Prenylamine (Segontin)

Diethylpropion (Amfepramone, Regenon)

Methoxamine (Vasoxyl)

Pargyline

Drazine

Nikethamide (Coramine)

Methylphenidate (Ritalin)

Using different extraction solvents and/or column conditions the following are also detected:

Narcotics such as morphine and heroin; other analgesics such as pethidine and methadone; stimulants such as caffeine, strychnine, leptazol etc; local anaesthetics such as lignocaine and many antihistamines .

Declaration of Results

Results of Phase 1 analysis (positives and negatives) were transmitted immediately by personal letter to the President of the Medical Sub-Committee at F.I.F.A. H.Q. The results were not made known to any other person.

After breaking the code, the President would then inform the laboratory if any positives reported were from players or from controls. In the latter case no further analysis would be undertaken. In the event of a sample from a player proving to be positive the following steps were scheduled.

- (i) Phase 2 and, if necessary, Phase 3 analysis of the sample in question would be undertaken.
- (ii) The President would notify the team officials involved that a 'prohibited drug' had been found on urine analysis. The name of the drug found would not be disclosed initially. If a satisfactory explanation for its presence in the sample in question were not forthcoming the Disciplinary Committee of F.I.F.A. would also be informed. In general, each case would be considered in

conjunction with the medical history of the player concerned e. g. the use of ephedrine as a bronchial dilator would constitute a defence if its use was adequately documented in the medical records of the player concerned.

- (iii) The Area Medical Officer and Sampling Officers who took the sample would be informed. If the team officials involved so desired, the duplicate sample kept by the Area Medical Officer would be further divided and the portions resealed in the presence of witnesses. One portion of this divided sample would then be given to the team officials for independent analysis.

Results

A total of 136 urine samples, of which 125 were from players, were analysed.

(a) Players' Samples:

All players' samples were reported as being negative for artificial stimulants.

(b) Control Samples:

The nine control samples were successfully identified. A further two blank urine controls were correctly reported as negative.

Appendix 1

Volume of Urine Samples

Urine samples collected from the players only occasionally exceeded 50 ml in volume and in many cases were as small as 5 to 10 ml. Although Phase 1 analyses could be done on less than 1 ml of urine if necessary, very small volumes, especially if they were to be further divided, could have produced difficulties in Phase 2 and Phase 3 analyses.

The problem of complete inability to micturate was approached in several ways including the drinking of large quantities of water or orange juice, listening to running water, taking a shower, whistling etc. Usually all that was required however, was a little time to allow the nervous tension built up during the game to subside.

In three cases the player was still unable to give a sample up to an hour after the game. However, since the players involved belonged to teams which had just been eliminated from the competition the matter was not pursued further.

DISCUSSION AND COMMENTS

The procedures in the first two phases of the analytical plan were based extensively on the use of gas-liquid chromatography. By the use of this technique and the preparation of derivatives of the drugs and their metabolites suitable for gas chromatographical examination, it is possible to establish the identity of the drug taken. (see Beckett, A.H. Tucker G.T., and Moffat A.C. - to be published in which retention times of drugs, metabolites and many derivatives on a variety of columns is presented). Thin-layer chromatography was used as a supporting technique, but, in general, it lacks the sensitivity and specificity of the gas-chromatographical procedures which have been adopted. If controversy on results between different laboratories occurs, an examination of the reserve urine sample in question can be made using the third phase of the analytical scheme, but in general this should not be required.

The sampling procedures adopted, the control of samples and the method of notification of results were intended to produce complete confidence among players, doctors and officials. If the presence of a drug in a player's urine had been established, then the procedures and controls used had to be sufficiently rigorous to withstand any potential

legal challenge.

The question of whether doping in professional football should be controlled or not is obviously not our primary concern, but there is sufficient evidence to indicate that drug-taking occurs. Our remit was to devise a rigorous sampling and testing procedure to indicate whether drugs were being taken by players during the World Cup series. The fact that tests were instituted, and knowledge became generally available that stimulants of the type which were forbidden could be detected in urine up to 30-40 hours after a dose, acted as a sufficient deterrent to ensure that drugs were not being taken by players immediately before and during matches.

We wish to place on record the wonderful co-operation we had from players and officials throughout this exercise on the use of a deterrent to control the misuse of drugs, a problem which is becoming increasingly evident in present day society.

Fig. 1 **Specimen Record Book Entry**

11th July **WEMBLEY** **ENGLAND v URUGUAY**

Player's Number	Player's Name	Team	Code	Signature of player to certify that the code on his bottles same as that in register	Signature of player to certify that the samples were sealed in his presence	Signature of F. I. F. A. official (Area M. O.) certifying that code on the bottles were the same as in the register	Signature of F. I. F. A. official (Area M. O.) certifying that samples were taken and sealed in his presence.
			111A				
			111B				
			111C				
			111D				

Signatures of Sampling Officers present:

- 1.
- 2.

Signature of Area Medical Officer present:

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LAB. DATA REPORT

[illegible]

Date Assay Completed:-
Results delivered to F.I.F.A. by:-
Instructions and messages for tomorrow -

ANALYST (Signature):-

WITNESS (signature):-

Table 3 **Identification of Control Samples**

Sample code Number	Compounds detected	Comments
313. E	1. Methylamphetamine 2. Amphetamine	Methylamphetamine ingested and amphetamine arising as metabolite of methylamphetamine.
215. E 316. C	1. Amphetamine 2. Phenmetrazine (Preludin)	
320. A	1. Ephedrine 2. Norephedrine	Ephedrine ingested and norephedrine arising as metabolite of ephedrine.
126. A.	1. Diethylpropion 2. Monoethylpropion 3. Propion	Diethylpropion ingested and monoethylpropion and propion arising as metabolites of diethylpropion.
126. B	1. Diethylpropion	Diethylpropion added to urine
126. F	1. Methylamphetamine 2. Amphetamine 3. Methylephedrine 4. Ephedrine 5. Norephedrine	Methylamphetamine ingested and amphetamine arising as metabolite of methylamphetamine. Methylephedrine ingested and ephedrine and norephedrine arising as metabolites of methylephedrine.
126. G	1. Norephedrine	
126. K	1. Methylamphetamine 2. Methylephedrine	drug added to urine drug added to urine
130. A	Blank	
130. B	Blank	

N. B. The detection of drug metabolites affords a method of distinguishing between the situation where the drug is ingested or otherwise administered and where it is simply added to the urine.