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BIOAVAILABILITY OF TETRACYCLINE, TETRACYCLINE HYDROCHLORIDE AND COMPLEXES

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The irregular and incomplete absorption of tetracycline from the GIT, directs the attention to use the organic complexes of this compound in therapy. Tetracycline complexes with urea, gentisic and pyrocatechuic acids were prepared and investigated in comparision with both tetracycline base and its hydrochloride salt. No correlation was found between the in-vitro results of lipophilicity of tetracycline base and its complexes and their availability. However, a correlation existed between the in-vitro $T_{50\%}$ dissolution of tetracycline base, tetracycline hydrochloride and its complexes in distilled water adjusted to pH2 , and their cumulative urinary excretion by human volunteers. The impaired absorption of tetracycline when adminstered with milk was found to be greatly reduced in case of tetracycline hydrochloride, while the absorption of tetracycline-pyrocatechuic and gentisic acids complexes were the least affected.

Gastrointestinal absorption of tetracycline base received much attention[1,2] since it is directly related to the biological activity of the drug and hence its absorption is surprisingly small compared to the total dose of tetracycline. The poor absorption of tetracycline is considered to be due to the polar structure of the tetracycline molecule [3] which in aqueous solution is almost entirely found in the cationic form under strongly acidic condition, anionic form under alkaline condition and zwitherinic form under the weakly acidic condition. It was also reported that, the ionization of tetracycline in the aqueous phase is a complex phenomenon and the resultant amount of the neutral form is small enough to be responsible for the low solubility of the tetracycline in the lipid phase [4,5]. However, it was recently reported by Uekama et al. [6] that the tetracycline molecule was mainly actively absorbed from the gastrointestinal tract through ion-pair formation. MacDonald et al. [7] suggested that, tetracycline is absorbed primarily in the upper intestinal tract, and implied that any drug which does not dissolve before

or during passage through the upper intenstinal tract remains unabsorbed. This hypothesis is compatible with Barr et al.[8] and Lovering et al.[9] data. According to MacDonald et al.[7], therefore, the bioavailability of tetracycline formualtions depends upon their rate of dissolution in the stomach and upper intestine and the rate at which the intestinal contents move through this region of the gastrointestinal tract. Lovering et al.[9] reported that, the relative rate of drug dissolution, absorption and passage through the absorptive segment of the intestine, determine the amount of drug absorbed from any oral dosage form. He also concluded that the dissolution rate, which was found to be strongly associated with the bioavailability, might be used as a tool assessing tetracycline formulations. On the other hand, Nelson[10] showed the existence of a rank order correlation between the rate of urinary exretion of four different forms of tetracycline and their corresponding in-vitro dissolution rates.

The objective of the present study was to test if a relationship existed between the urinary excretion of tetracycline base, hydrochloride and tetracycline complexes with urea, gentisic andpyrocatechuic acids and their in-vitro dissolution behaviour and lipophilicity.

EXPERIMENTAL

I Materials

Tetracycline hydrochloride (El-Nasr Co.), tetracycline base (El-Nasr Co.), tetracycline-urea complex [11], tetracycline-gentisic acid complex [12] and tetracycline-pyrocatechuic acid complex [12] were the drugs used in the present study.

Citric acid (BDH), disodium hydrogen phosphate (BDH), sodium chloride (BDH), n-octanol (Problabo), hydrochloric acid (BDH), sodium hydrexide (BDH) .and sodium nitrite (BDH) were also used.

II Equipment

The equipments used were: a rotating bottle apparatus (N.F. XII), a pH-meter (Radelkis, type OP-20411), a spectrophotometer (Spektromom, type 203), a ball-mill (Labor, type W.J.), a set of sieves (VED) and vibrating shaker (Labor, type ILM) and a projection microscope (type PZO, MP3).

III Procedures:

1. Particle Size Determination

The average particle size of tetracycline base and tetracycline complexes with urea, gentisic acid and pyrocatechuic acid samples used in the dissolution rate and bioavailability studies, were determined using a projection microscope. The statistical average particle size of each sample was calculated from the measurement of up to 1000 particles per single mount [13]. Tetracycline and its complexes with urea, gentisic and pyrocatechuic acids were used to have more or less the same statistical particle size of 12, 11, 13 and 11 microns respectively.

2. In-Vitro Studies

A. Apparent Partition Coefficients Determination

Exactly the equivalent of 5.203×10^{-6}M and 2.6×10^{-6}M of tetracycline base from both its hydrochloride salt and complexes respectively were weighed and dissolved in 100ml of buffer solutions. The apparent partition coefficient of tetracycline molecules between n-octanol and aqueous McIlvaine's buffer [14], adjusted to 0.1 ionic strength at pH values of 2.2, 5.6 and 7.4, were determined. This was carried out by rotating equal volumes of the two phases (10 ml each) in glass stoppered bottles immersed in a water bath previously adjusted to 37^{o} for one hour [15], a time which was found, experimentally, to be quite sufficient for achieving equilibration of tetracycline molecules between the two phases. The concentration of the antibiotics in the aqueous phase was determined spectrohotometrically in 0.1 N HCI solution at 353 nm [16].

B. Dissolution-Rate Measurements

The apparatus used for the dissolution rate measurement was the rotating bottle apparatus, official in the National Formulary XII [17]. From each of the materials under investigation (tetracycline base, tetrcycline-urea, tetracycline-gentisic acid and tetracycline-pyrocatechuic acid complexes that having the statistical average particle size of 12, 11, 13 and 11 microns respectively) exactly 200 mg was transferred to a glass stoppered tube. Twenty ml aliqots of distilled water, adjusted to different pH values, were added to each tube and arranged in the rotation shaft to rotate at 50 r.p.m . At various time intervals (5, 10, 15, 20, 25, 30 and 40 minutes) 1 ml samples were withdrawn from each bottle with a 1 ml-pipette fitted with a filtering sintered glass device. The samples were suitably diluted with 0.1 N HCI and their tetracycline content was determined spectrophotometrically at 355 nm using an appropriate blank.

From the dissolution rate data, the time required for the 20% dissolution of the materials under test, at different pH values, were determined. A further determination of $T_{50\%}$ dissolution of the tetracycline compounds at pH2 was also carried out.

3. In-Vivo Studies

Five adult volunteers, with no known diseases, of body weight ranging from 53 to 90 kgm and between 40 and 50 years in age, were served as subjects. Each volunteer was screened regarding general health and any know drug allergies. All subjects were instructed to adhere to the standard protocol and abstained from taking any medication, including vitamins, during the course of study. Each subject was given a single dose of tetracycline base and its hydrochloride salt and complexes equivalent to 3 mg of the base per kilogram body weight in capsule form. The dose was taken in the morning, one hour before a standard

breakfast free of any milk products. A blank urine samples was collected prior to taking the dose, and urine samples were collected at 2, 4, 6, 8, 10 and 12 hours after administration of the dose. The total volume of the sample was recorded along with the exact time of collection. The samples were transferred to glass stoppered bottles and refrigerated until analysed. Effect of administration of milk on the urinary excretion of tetracycline base, hydrochloride and complexes was also investigated following the same procedure. Mortada et al. Colorimetric method [18] was employed for the determination of the amount of tetracycline and its complexes excreted in the urine.

RESULTS AND DISCUSSIONS

1. Urinary Excretion of Tetracycline Compounds as a Parameter of their Bioavailability

Nelson[19] and Nelson and Schaldemose [20] pointed out the value of urinary excretion kinetics for evaluation of rate of drug absorption. Shenoy [21] published data on the urinary excretion rate and bioavailability measured in man. Barr et al, [22] found that the bioavailability of tetracycline could be calculated from urinary excretion data, collaborating earlier work by Chulski et al. [23]. Therefore, in the present study the bioavailability of tetracycline base, hydrochloride and its complexes was calculated from the urinary excretion data.

Comparing the lipophilicity of tetracycline hydrochloride and tetracycline complexes as a measure of their passive diffusion through the G.I.T. with their bioavailability (Figures 1 and 3) it could be concluded that, tetracycline is not mainly absorbed by passive diffusion mechanism which depends on their lipophilicity. Therefore, it could be assumed that tetracycline is actively absorbed mainly. This is in agreement with the findings of Klink and Colaizzi [24] and Uekama et al [6].

According to MacDonald et al.[7], tetracycline is absorbed primarily in the upper intestinal tract and that, any drug which does not dissolve before or during passage through the upper intestinal tract remains unabsorbed This hypothesis is compatible with the findings of other authors [8, 9, 25]. As the bioavailability of tetracycline formulations depends upon their rate of dissolution in the stomach and intestine [9], trials were made, in the present study, to find out any correlation that may exist between the dissolution time ($T_{50\%}$) of the tetracycline compounds in distilled water (adjusted to pH2) and the in-vivo availability of the antibiotic. The selection of $T_{50\%}$ dissolution in distilled water ajusted to pH2, simulating that of the stomack juice, is based on the findings of MacDonald et al.[7].

The effect of pH variation of the dissolution medium (distilled water) on the time required for the 20% dissolution of tetracycline base and its complexes with urea, gentisic and pyrocatechnic acids is illustrated in Figure 2. From Figure 2, it is obvious that at pH2 the highest dissolution of the materials under investigation was obtained. It could also be observed that from the tetracycline compounds tested, tetracycline-pyrocatechnic acid complex is the least compound affected by the variation of pH value of the dissolution medium followed by gentisate and urea complexes and tetracycline base.

From Figure 4, it could be noticed that the maximum urinary excretion of the antibiotic occurs after two hours if ingesting tetracycline-gentisic acid complex and four hours for tetracycline hydrochloride, urea and pyrocatechnate complexes, and six hours upon ingesting tetracycline base. The cumulative urinary excretion of tetracycline compounds after twelve hours of ingesting the does is graphically illustrated by Figure 4. Figure 4 shows that tetracycline hydrochloride is the most highly excreted compound followed in a decreasing order by pyrocatechnate complex, tetracycline base, urea complex and gentisate complex. However, the eight hours cumulative

urinary excretion of the antibiotics under investigation was found to correlate well with the time required for 50% dissolution of the powders having more or less the same particle size. Figure 5, is a scatter diagram of the relative cumulative urinary excretion (0-8 hours) versus the relative time of 50% dissolution of the compounds at pH2 which shows direct correlation between the two parameters.

2. Effect of Administration of Milk on the Urinary Excretion of Tetracycline Base, Hydrochloride and Complexes

Many reports were published dealing with the agents which increase [26, 27] or decrease [28, 29] the absorption of tetracycline in man. Banerjee and Chakrabarti [30] stated that in the presence of certain cations, like calcium, magnesium and iron in the mucosal fluid, a significant decrease in the transport of tetracycline through everted mice ileum takes place. These observations mirror those in man [28, 31]. Generally, absorption of tetracycline is greatly reduced by calcium, magnesium and aluminium containing antacids and by iron preparations [1]. Foods, especially milk products or other high calcium foods, also interfere with oral absorption of tetracycline. Phosphate appears to improve absorption rate by removing calcium [1].

Table 1 illustrates the amount of tetracycline excreted after 2,4,6,8,10 and 12 hours of ingesting dose equivalent to 3 mg/kgm body weight of the compounds under investigation. From the table, it is clearly obvious that the cumulative urinary excretion of tetracycline is highly reduced in case of tetracycline hydrochloride. The least reduction in the cumulative urinary excretion was observed in case of using tetracycline-gentisic acid and pyrocatechuic acid complexes. This could be explained in view of the well know fact that tetracycline-HCl is the more soluble compounds of the materials under investigation, and subsequently the interaction between the soluble tetracycline hydrochloride and alkali earth metals is high.

CONCLUSIONS

1. The in-vitro results of lipophilicity of tetracycline base and its complexes were not found to correlate with their bioavailability. This leads to the conclusion that tetracycline is not absorbed from the gastro-intestinal tract by the passive diffusion mechanism.

2. Good correlation existed between the in-vitro $T_{50\%}$ dissolution of tetracycline base, tetracycline hydrochloride and its complexes in distilled water at pH2 and their cumulative urinary excretion after eight hours of ingesting the same dose of each compound. Therefore, the dissolution rate of the antibiotics under investigation is the rate limiting factor for their absorption, and this is the case in which the drug is mainly actively absorbed.

3. The freely soluble tetracycline hydrochloride was found to be the most highly excreted compound followed in decreasing order by pyrocatechuate complex, tetracycline base and gentisate complex.

4. The impaired absorption of the tetracycline when co-administered with milk was found to be greatly reduced in case of tetracycline complexes. Tetracycline-pyrocatechuic and gentisic acid complexes being the least affected ones.

TABLE 1. Human Urinary Excretion of Tetracycline Hydrochloride (A), Tetracycline Base (B), Tetracycline-Urea Complex (C), Tetracycline-Gentisic Acid Complex (D) and Tetracycline-Pyrocatechuic Acid Complex (E) ; after Ingesting the Equivalent Doses with Water and Milk.

Subject	Compound	Cumulative Amount of Free Tetracycline Excreted in Urine (in mg.) after:-											
		2 h.		4 h.		6 h.		8 h.		10 h.		12 h.	
		water	milk	water	milk	water	milk	water	milk	water	milk	water	milk
1	A	8,70	1,65	16,09	1,69	22,29	2,83	26,80	13,29	31,66	16,20	33,66	16,99
	B	3,56	2,62	11,62	8,23	19,72	8,42	27,36	13,31	30,37	16,12	31,88	17,32
	C	2,09	1,09	11,93	7,43	18,95	12,51	23,12	15,99	26,02	18,96	27,10	20,93
	D	11,75	6,52	22,92	12,92	32,38	17,90	37,54	25,98	40,87	29,11	44,84	31,21
	E	9,62	7,45	17,89	12,51	24,39	16,08	29,87	18,39	35,27	20,55	38,63	25,63
2	A	4,89	2,64	26,03	16,07	41,27	26,44	50,76	34,54	57,14	39,52	62,13	41,67
	B	2,76	1,75	13,15	6,32	25,64	14,07	33,71	20,05	37,82	29,44	50,35	34,13
	C	4,30	3,33	9,03	7,47	22,71	18,83	32,54	25,31	44,38	32,04	51,63	36,54
	D	6,98	2,89	13,71	6,74	18,64	13,54	20,96	15,66	22,31	16,82	25,09	17,48
	E	11,06	10,80	23,90	20,14	30,36	24,66	36,54	27,02	40,61	29,36	44,52	31,22
3	A	7,19	3,82	21,52	10,82	39,59	18,25	55,91	22,08	63,05	31,60	74,77	35,48
	B	5,44	2,97	13,61	9,32	21,20	13,72	27,96	17,12	31,98	19,14	34,92	20,45
	C	1,75	0,91	10,35	6,43	15,71	11,01	23,27	15,94	27,28	17,89	34,93	25,86
	D	10,52	6,18	15,14	8,95	19,33	14,02	21,81	18,35	25,11	20,13	27,09	18,14
	E	6,92	5,23	17,34	14,79	28,34	22,92	36,77	28,01	42,58	29,36	44,63	31,08
4	A	6,70	2,99	13,16	4,64	18,10	7,43	31,77	7,98	40,19	12,06	44,33	15,59
	B	3,55	1,96	9,51	6,12	20,64	12,99	25,24	16,63	28,98	18,45	32,39	19,98
	C	3,14	1,80	12,09	7,97	19,21	12,35	24,27	16,85	26,34	17,06	26,99	17,99
	D	10,45	7,12	18,62	13,40	25,99	19,99	31,21	24,26	36,34	29,61	39,36	30,38
	E	12,95	8,71	18,31	14,78	21,78	19,19	24,02	21,50	25,90	23,40	26,81	24,21
5	A	6,70	3,78	19,57	11,32	32,69	12,86	42,05	13,80	51,79	17,08	64,30	20,99
	B	1,35	0,96	5,96	4,80	14,91	11,92	21,74	15,20	22,83	17,01	23,20	17,37
	C	1,68	0,69	8,27	5,41	9,57	7,04	12,42	10,67	14,31	12,42	15,51	13,01
	D	7,12	6,01	13,42	10,67	19,49	13,15	20,48	14,96	21,72	15,72	22,03	16,18
	E	3,61	2,93	12,17	9,02	8,19	14,06	24,32	18,02	27,32	22,05	29,43	23,13

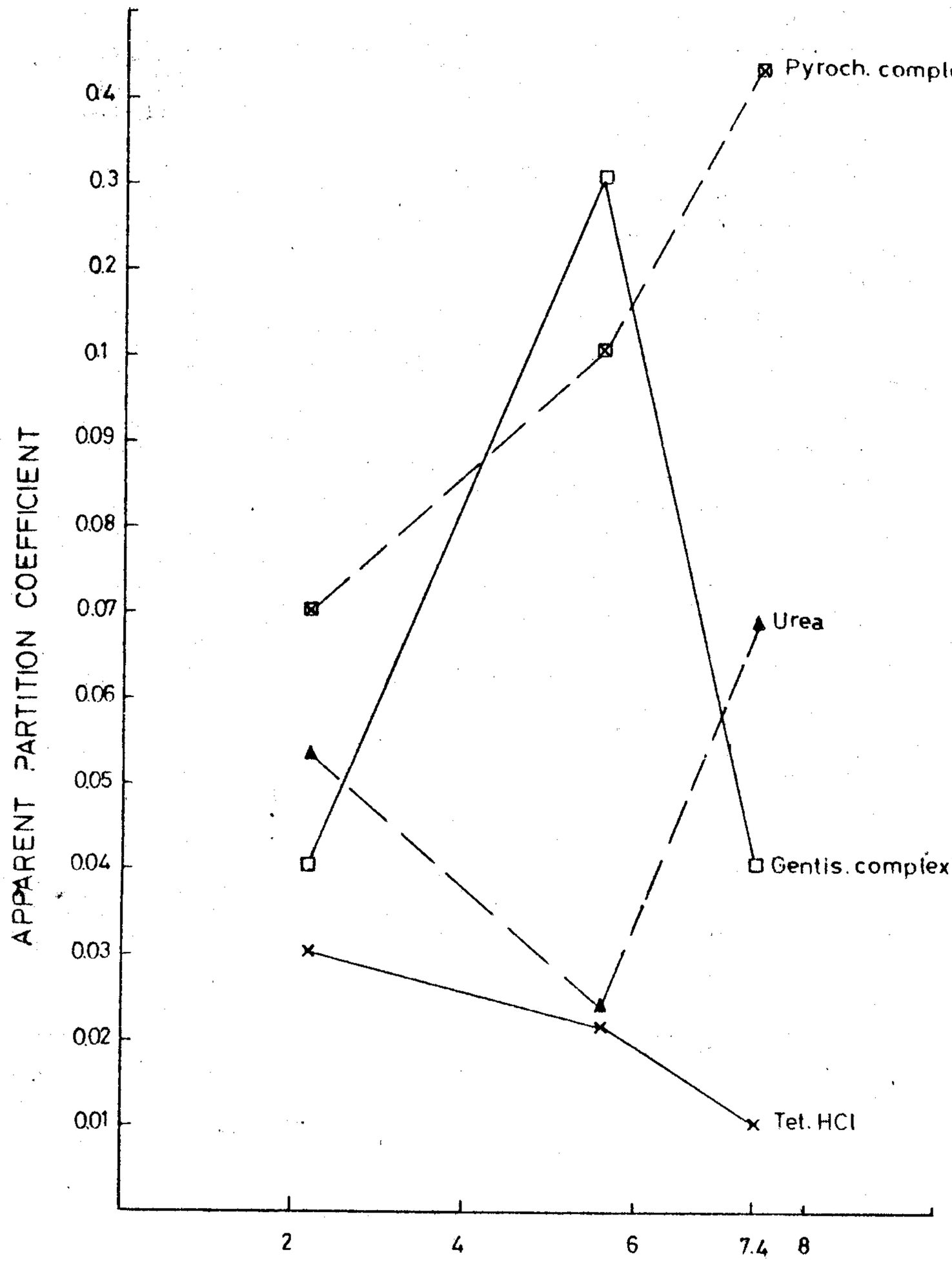

FIG.(1:) INFLUENCE pH VALUES ON APPARENT PARTITION COEFFICIENTS OF TETRACYCLINE BETWEEN N-OCTANOL AND AQUEOUS McILVAINE'S BUFFERS ADJUSTED TO 01 IONIC STRENGTH WITH NaCl AT 37°C.

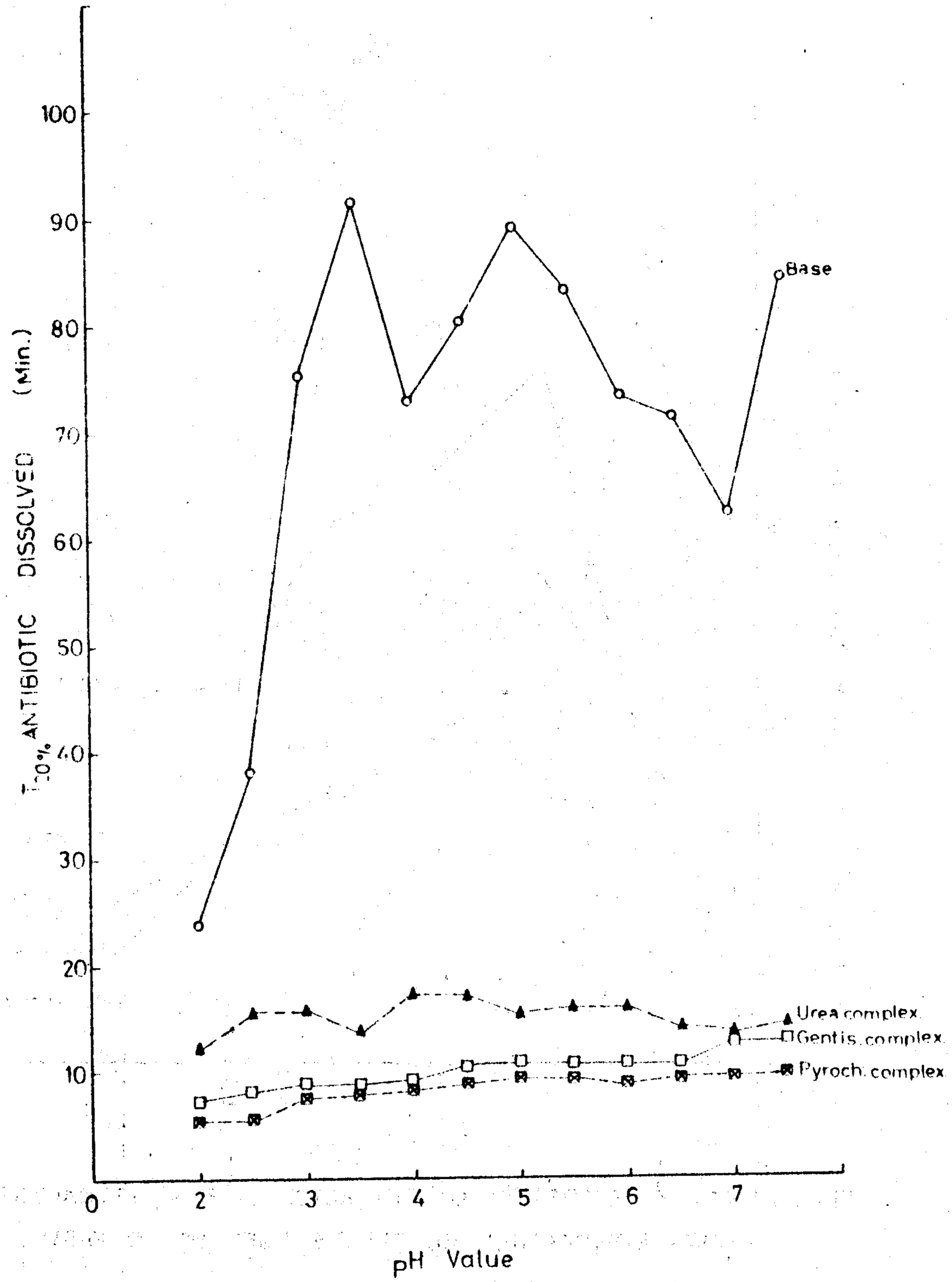

FIG.(2) $T_{20\%}$ DISSOLUTION OF TETRACYCLINE BASE AND ITS COMPLEXES IN DISTILLED WATER ADJUSTED TO DIFFERENT pH VALUES, AT 37°C.

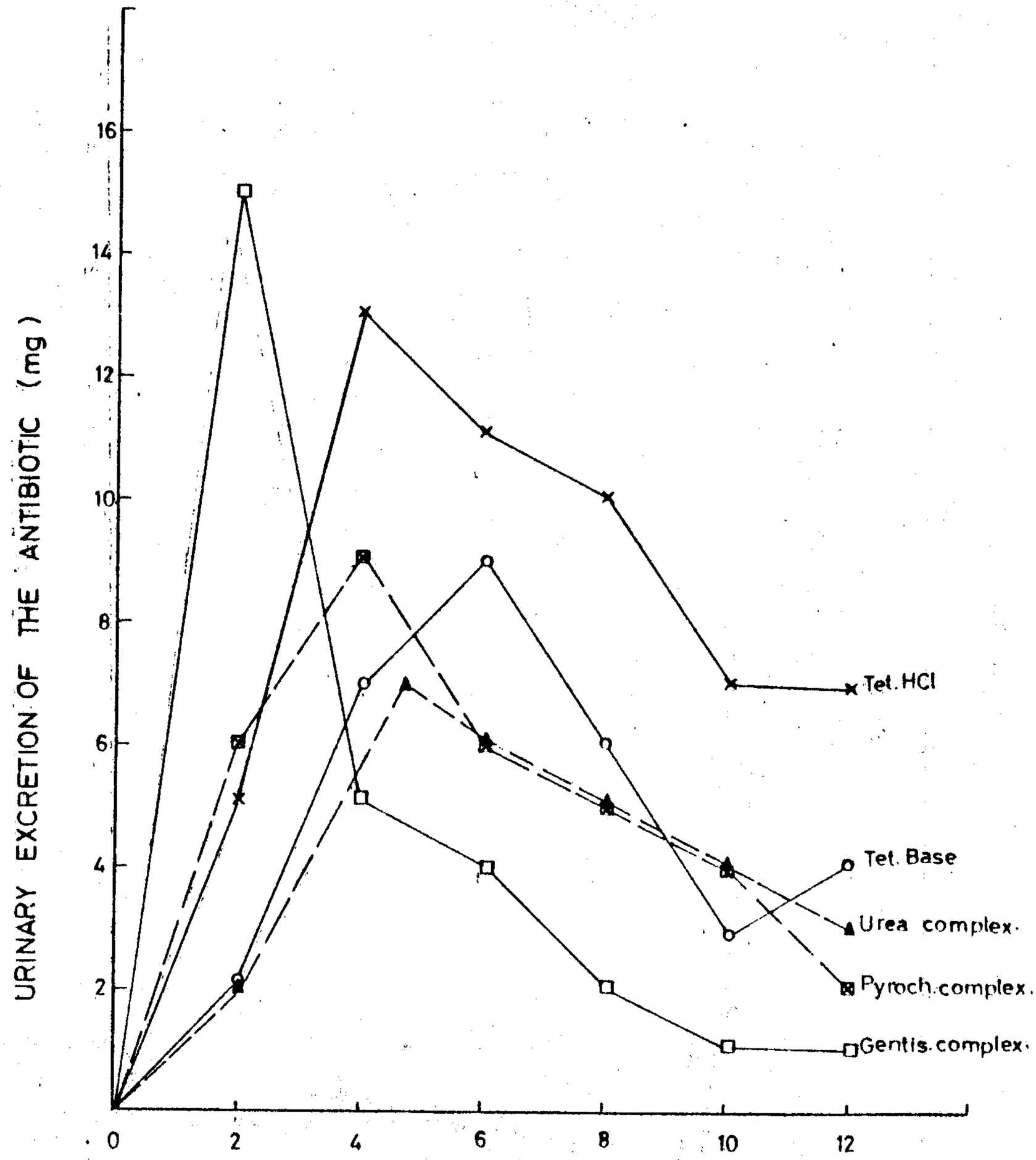

FIG.(3) URINARY EXCRETION OF TETRACYCLINE BASE, TETRACYCLINE HYDROCHLORIDE AND COMPLEXES INGESTED TO FASTED HUMAN VOLUNTEERS.

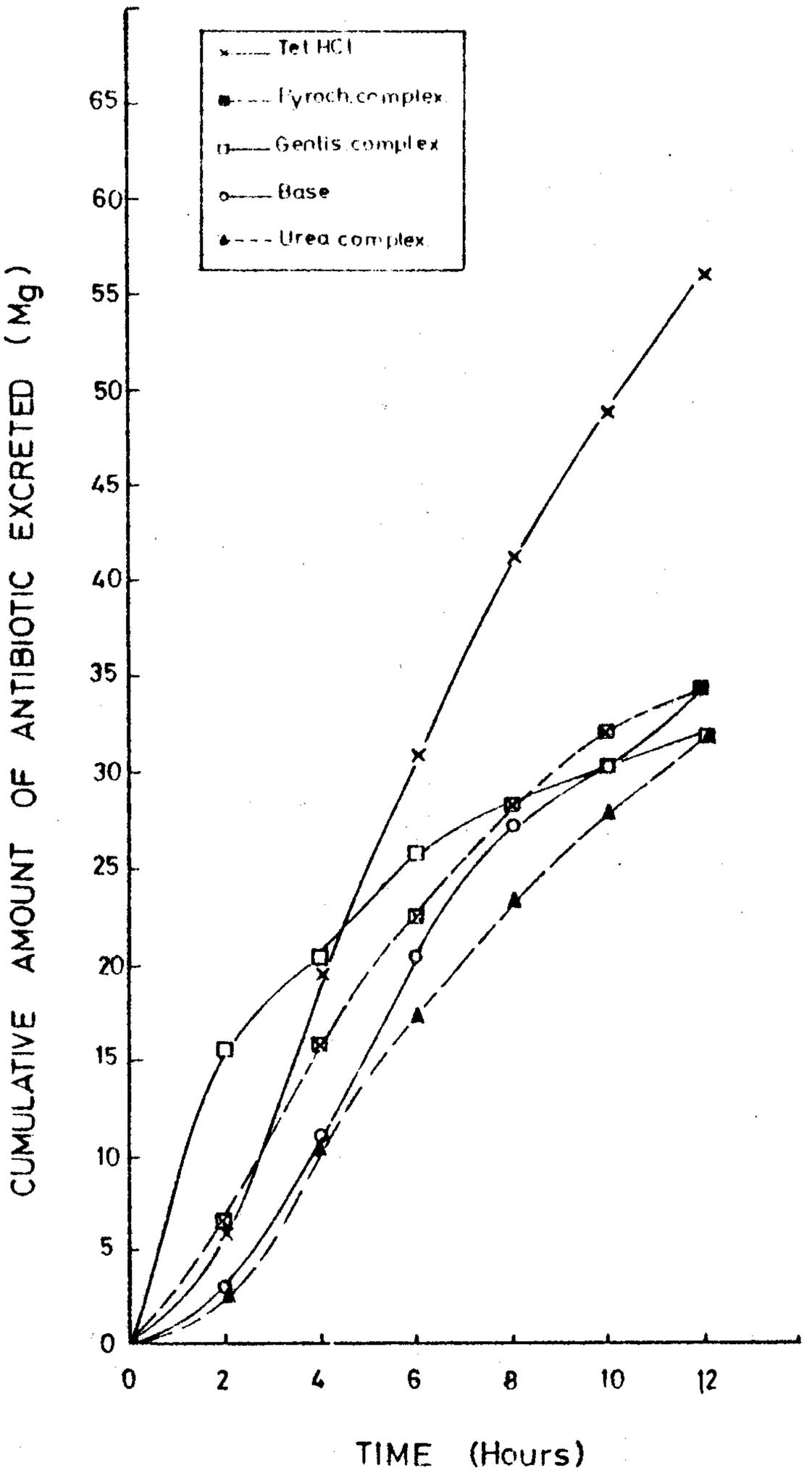

FIG.(4) CUMULATIVE URINARY EXCRETION OF TETRACYCLINE BASE, TETRACYCLINE HYDROCHLORIDE AND COMPLEXES INGESTED TO FASTED HUMAN VOLUNTEERS.

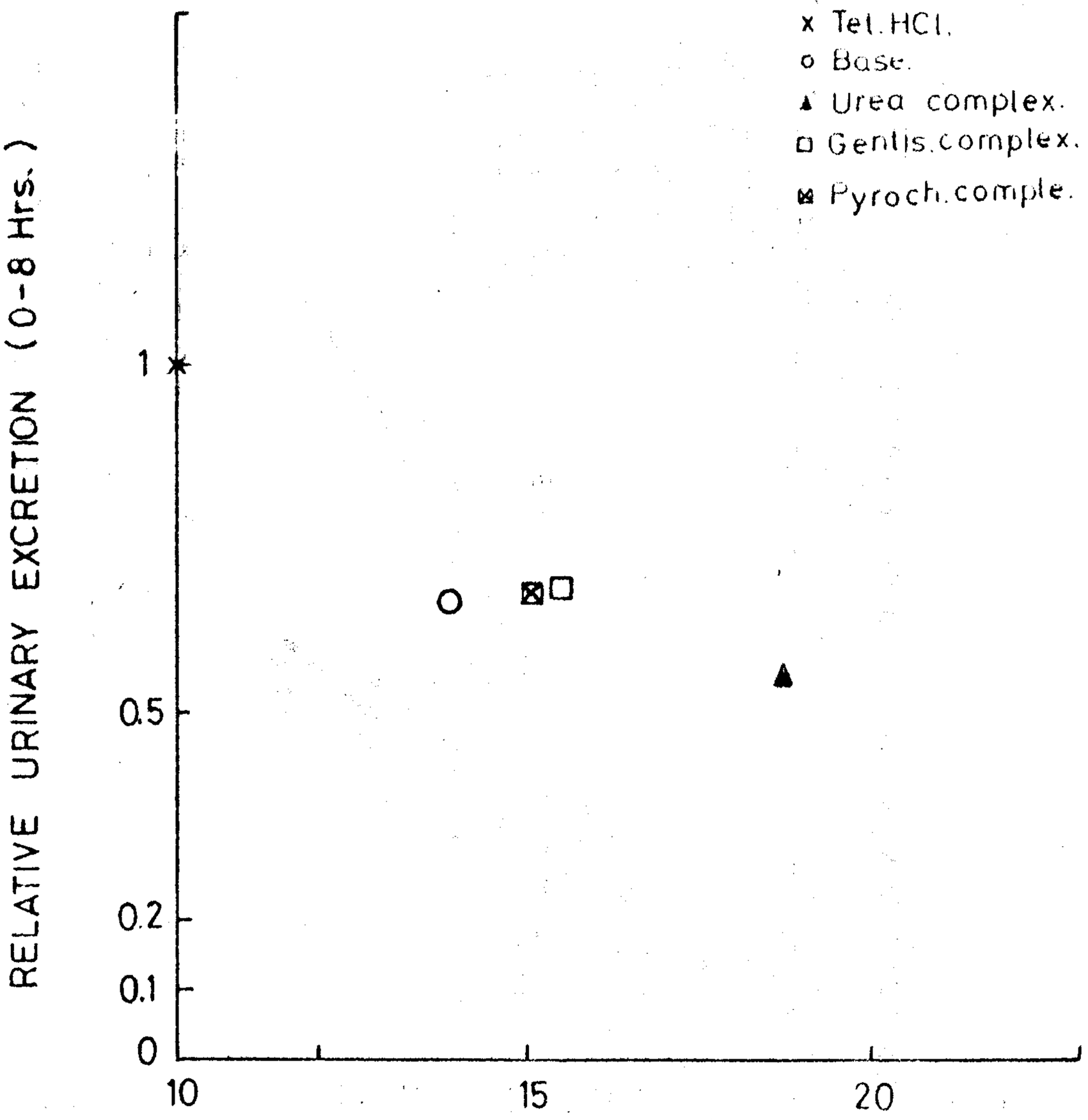

FIG.(5) SCATTER DIAGRAM OF THE EXCRETION (0-8 Hrs)

VERSUS THE RELATIVE $T_{50\%}$ DISSOLUTION OF

TETRCYCLINE BASE, HYDROCHLORIDE AND COMPLEXES

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التوائر الحيوى للتتراسيكلين وملحه الايدروكلوريدى وبعض متراكباته

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لقد كانت ظاهرة عدم أنتظام أمتصاص التتراسيكلين من الجهاز الهضمى الحافز لدراسة احتمال استعمال المتراكبات المضيفه لهذا المضاد الحيوى فى العلاج فحضرت ثلاث متراكبات التتراسيكلين مع اليوريا وحمض الجنتزيك وحمض الهيروكا تشيويك حيث أجريت دراسة معمليه وحيوية لايجاد العلاقه بين نتائجهما وجد أنه لا يوجد علاقه بين الدراسات المعمليه التى تختص بدراسة قابلية قاعدة التتراسيكلين ومتراكباته للذوبان فى الدهون وتوافرها الحيوى . فى حين أنه قد وجدت علاقه تربط بين نتائج الدراسات المعمليه من حيث الوقت اللازم لاذابة ٥٠ فى المائه من مساحيق قاعدة التتراسيكلين وملحه الايدروكلوريدى ومتراكباته فى ماء مقطر قيمته الايدروجينيه ٢ وبين كمية المضاد الحيوى التى تفرز فى البول على مدى ثمانى ساعات من بلع كبسولات تحتوى على جرعات متكافئه من مساحيق موحدة الجرم .

ودراسة تأثير تناول اللبن مع التتراسيكلين ومتراكباته على توافرها الحيوى وجد أن التأثير المعطل للبن لامتصاص التتراسيكلين ينخفض انخفاضا كبيرا بتناول متراكبات التتراسيكلين حيث أظهر كل من متراكبى الهيروكا تشيوات والجنتيزات انهما أقل المضادات الحيويه المستخدمه تأثيرا بتناول اللبن .