1963/169
B

Copy for Chief Geophysicist

COMMONWEALTH OF AUSTRALIA.

DEPARTMENT OF NATIONAL DEVELOPMENT.
BUREAU OF MINERAL RESOURCES
GEOLOGY AND GEOPHYSICS.

RECORDS:

1963/169

SYNGENESIS OF SULPHIDE ORES; SULPHATE REDUCING BACTERIA AND COPPER TOXICITY

by

K.L.Temple and N.W.le Roux

SYNGENESIS OF SULPHIDE ORES: SULPHATE-REDUCING BACTERIA AND COPPER TOXICITY

by

K.L. Temple and N.W. le Roux

RECORDS 1963/169

CONTENTS

SYNGENESIS OF SULPHIDE ORES: SULPHATE-REDUCING BACTERIA AND COPPER TOXICITY[1]

K.L. TEMPLE[2] AND N.W. LE ROUX[3]

Records 1963/169

ABSTRACT

Some syngenetic theories of sulphide ore formation involve micro-organisms as sulphate-reducing agents. The toxicity of copper has constituted an objection to syngenesis of copper sulphides. The effect of copper sulphate on sulphate-reduction by Desulfovibrio was followed manometrically and checked by chemical analysis. Copper toxicity was evidenced by an excess of copper sulphate over that required to precipitate all the sulphide. Sulphate-reducing cultures were protected from copper toxicity to the extent that they produced sulphide. Copper sulphate concentrations of 0.25 to 0.29 percent were required to inhibit sulphate-reduction. Copper toxicity is consequently not a serious factor in the problem of syngenesis.

INTRODUCTION

A biochemical source of sulphide is an integral part of some current theories of the syngenesis of conformable metal sulphide ores. These controversial theories have been attacked on various grounds. This paper considers only one of the most serious objections, i.e. the toxicity of copper ions (2, 3). The precipitation of copper sulphide in a sedimentary basin through the action of sulphate-reducing bacteria could not long continue, if copper were sufficiently toxic to prevent biological sulphate-reduction. Sulphate-reducing bacteria of the genus Desulfovibrio are the only known organisms sufficiently ubiquitous and active to be responsible for sulphate-reduction on the requisite scale. Cuprous and cupric salts are generally toxic to bacteria, but no specific information exists on the effect of copper on Desulfovibrio. Copper was selected for this study because of the large mass and extent of copper sulphide ores that are claimed to be syngenetic and because of the specific references to copper toxicity by opponents of syngenesis. The experiments reported examine the effect of copper sulphate on Desulfovibrio cultures and their ability to precipitate copper sulphide.

---

[1] Published with the permission of the Director, Bureau of Mineral Resources, Geology and Geophysics, Canberra and the Chief, Division of Plant Industry, C.S.I.R.O., Canberra.

[2] Division of Plant Industry, C.S.I.R.O., Canberra. Present address Department of Botany and Bacteriology, Montana State College, Bozeman, Montana.

[3] Bureau of Mineral Resources, Geology and Geophysics, Canberra.

RESULTS OF NEW WORK

Source of Sulphate-Reducing Bacteria

The Desulfovibrio culture was obtained from Pernatty Lagoon, an inland salt lake in South Australia. The thin salt crust at the point of sampling was stained by copper salts that had leached from a near-by copper tailings pile. Beneath the salt crust was a discontinuous black zone with a sulphide odour. This black mud was inoculated into the Desulfovibrio medium described below and was repeatedly subcultured. The result was a dense population of sulphate-reducing bacteria corresponding morphologically to the genus Desulfovibrio, and the culture was not further purified.

Experimental Method

Bacterial medium, manometric measurements and chemical analysis

Desulfovibrio can use molecular hydrogen (H_2) to reduce sulphate to sulphide according to the reaction:

$$SO_4^{=} + 4H_2 \ \underline{\qquad\qquad}\ S^{=} + 4H_2O$$

This reaction suggested a simple means of following the course of sulphate reduction without disturbing the system. Replicate culture flasks were connected to a H_2 reservoir, a manometer and levelling bulbs (Fig.1). Gas volume changes were measured at constant temperature (30°C) and pressure (69.95 cm Hg). Cupric sulphate was added to individual flasks as desired. The cultures consisted of fully-grown populations of Desulfovibrio in a medium of 0.05% K_2HPO_4, 0.05% $(NH_4)_2SO_4$, 0.05% $MgSO_4.7H_2O$, 0.3% Na lactate, 3.0% NaCl and 0.1% of a solution of trace elements. The trace element solution consisted of 360 mg% $FeCl_3 6H_2O$, 57 mg% H_3BO_3, 44 mg% $ZnSO_4. 7H_2O$, 20 mg% $CoCl_2.6H_2O$, 2 mg% $CuSO_4. 5H_2O$, 2 mg% $MnCl_2.4H_2O$ and 5 mg% $Na_2MoO_4. 2H_2O$. Desulfovibrio uses both molecular H_2 and the hydrogen from lactate when these are present at the same time. Only H_2 uptake was measured. On completion of the manometric measurements, copper and sulphur distribution analyses were made. Volatile sulphide was washed into an ammoniacal cadmium chloride trap by argon gas. The medium was then filtered into ammoniacal cadmium chloride, separating the copper sulphide (on the filter) and the dissolved sulphide (as CdS precipitate). The CdS was filtered and the sulphate remaining in solution was precipitated with barium chloride. Sulphides were oxidized by nitric acid and bromine water to sulphate and all sulphates determined gravimetrically as $BaSO_4$. Copper was determined colorimetrically using the 2:2' diquinolyl method (4).

Addition of Copper Sulphate to Desulfovibrio Culture

The amount of copper sulphate used (chosen on the basis of preliminary experiments) ranged from 0.0062 to 0.6242 grams $CuSO_4. 5H_2O$ per 250 ml. of medium, equal to 10^{-4} to 10^{-2} molar copper at each addition. The time of addition was chosen to determine approximate threshold toxicity concentrations and the protective action of sulphide. The addition of different amounts of copper and the accumulative effect of repeated additions were also designed to elucidate these factors. Details of the copper sulphate treatments are best visualized by referring to figure 2. The arrows indicate the time of addition and the number of bars on the arrows indicate the amount of copper added. Total amounts are included in table II.

H_2 uptake and Copper Toxicity.

A distinct series of conditions emerged from the five experimental flasks. Flask 2 received no copper, while flask 4 had a lethal dose. Flasks 3, 5 and 1 had increasing sublethal amounts of copper respectively. More detailed results follow.

(Flask 2) Curve 2 (fig.2) shows H_2 uptake in the absence of copper. The rate dropped abruptly after nine days as the sulphate was exhausted. Table I records a 98.9% reduction of sulphate in this flask.

(Flask 4) H_2 uptake in flask 4 ceased entirely after eight days, due to copper toxicity. This unit had received two additions of copper with no effect on the rate of H_2 utilization but the third addition at six days of 0.6242 grams $CuSO_4.5H_2O$ stopped bacterial action. Analysis showed only 36.8% sulphate-reduction (Table I) and appreciable free copper ion in solution (TableII). A growth test showed that the bacteria were dead.

(Flask 3) Two treatments of 0.1248 grams $CuSO_4.5H_2O$ had no adverse effect on gas uptake by flask 3 and the curve approximated a straight line for the entire three weeks. Soluble copper was not detectable in this unit and 85% of the sulphate was reduced to sulphide.

(Flask 5) Five copper additions were made to flask 5. The final treatment was followed by a temporary decrease in H_2 uptake rate with complete recovery four days later. The sulphate reduction of 73.3% in the flask confirms the bacterial activity indicated by H_2 uptake.

(Flask 1) Flask 1 had the highest initial gas uptake rate. It received a single treatment of copper sulphate equivalent to 0.249% $CuSO_4$. $5H_2O$. This massive dose dramatically slowed the sulphate-reducing action as shown by the H_2 uptake curve, but recovery, after twelve to thirteen days, was complete. The extended period of low activity was reflected in the 46.5% sulphate-reduction.

The H_2 uptake results may be summarized briefly. Complete inhibition of sulphate-reduction by Desulfovibrio required the addition of enough copper sulphate to give between 0.249 and 0.289 percent solution. Temporary inhibition without destroying the bacteria resulted from copper concentrations of 0.129 to 0.249 percent. Lesser concentrations had no effect except that of supplying additional sulphate and of precipitating copper sulphide.

DISCUSSION

Experimental Design

This experiment was not a test of copper toxicity in the ordinary sense. Such an experiment could have been done, perhaps by harvesting a mass of Desulfovibrio cells and testing their ability to reduce sulphate, to use H_2 or organic matter in the presence of various levels of cupric ion, or by investigating their ability to grow after exposure to copper salts. The whole point of this paper is that the obvious practical aspects of copper toxicity for Desulfovibrio have previously been ignored. Sulphate-reducing cultures are bathed in a zone of sulphide which they constantly replenish. A priori, one would expect that the copper salts reaching this zone would be precipitated at its periphery and that this precipitation would be an effective protective mechanism against copper toxicity.

From this viewpoint, the experimental design was a severe test. Copper sulphate was added directly to a small volume of culture (250 ml) so that the precipitate copper sulphide was in close proximity to the bacteria. The experiment tested the capacity of the bacteria to protect themselves by precipitating copper ions as copper sulphide. The experiment determined the practical limits of the protective mechanism.

Both cuprous and cupric ions are toxic. Cuprous salts are readily oxidized and are less soluble than cupric, therefore less useful for postulating transport in aqueous systems. Cupric sulphate is stable in the medium used if sulphide is absent. Both cuprous and cupric ions form insoluble sulphides. The mineralogical identity of the precipitate is not relevant to the problem of toxicity. The precipitate evidently consisted of mixed copper sulphides since the Cu:S ratio was variable.

The Pernatty Lagoon culture was originally chosen because it initiated growth in media with a higher copper content than did an estuarine culture. However, this difference was trivial in comparison to the protective action that resulted from reducing sulphate to sulphide.

The design of the experiment, although artificial in comparison with a sedimentary basin, was realistic. It furnished a model of an active sulphate-reducing environment to which copper salts might be added according to plan. The criterion of copper toxicity was a slowdown or ceasation of sulphate-reduction. This is the criterion which is most relevant to a natural environment and the problem of sulphide ore formation.

Meaning of Results

The principle which emerges from this study is that a sulphate-reducing organism is protected from heavy metal ion toxicity by the sulphide it produces. This principle would apply to any biological sulphate-reducing agent and to any metal that formed an insoluble sulphide. More specifically, the relation of copper toxicity to biogenesis of copper sulphide does not depend upon the inherent toxicity of cupric or cuprous ions for Desulfovibrio cells. It depends upon the relative rates of two processes. These processes are the influx of copper salts and the production of sulphide from sulphate.

The results plainly show the sulphate-reducing bacterial cultures are unaffected by copper unless the amount of copper added exceeds the amount necessary to precipitate all of the sulphide present. The toxic level demonstrated here, of about 0.25% copper sulphate, is very high and allows ample freedom for theories of ore genesis involving copper-enriched waters. In an actual sedimentary basin, short, discontinuous bursts of much higher copper concentration could be tolerated. Bacterial sulphate-reduction could be stopped only by an amount of copper more than sufficient to react with all of the sulphide in solution. A sudden flood of copper-enriched water less than this amount would leave some bacteria alive and active and able to repopulate the entire anaerobic area of the basin. On the other hand, steady supply of 0.1 percent copper solution (non-toxic in this experiment) would overwhelm a sulphate-reducing culture that was limited spatially or by scarcity of a hydrogen or a sulphate source.

Assuming a lethal copper concentration of 30 p.p.m. Davidson estimated that copper-enriched waters would have a sulphate-S to Cu ratio of at least 30:1. (3). Our data show that the amount of copper tolerated is governed only by the amount of sulphide present.

It is not the purpose of this paper to advocate syngenetic theories or to assess them in toto. There has been considerable speculation on the toxicity of copper as an argument against syngenesis. The present paper considers only this argument. Our evidence suggests that copper toxicity is not a strong argument against the syngenetic theory of copper sulphide formation. This conclusion should apply to any similar organism existing in earlier geological times and to the other heavy metals.

SUMMARY AND CONCLUSIONS

1. Copper sulphate was added to cultures of sulphate-reducing bacteria (Desulfovibrio) in varying amounts over a three-weeks period. The effect on sulphate-reduction was followed by daily measurements of H_2 uptake and by chemical analysis on completion of the experiment.

2. The chemical analysis confirmed the interpretation of bacterial activity and copper toxicity from H_2 uptake data.

3. Ionic copper in a concentration of 3.0 mg Cu/liter sterilized the culture and stopped sulphate-reduction. A Cu^{++} concentration of 0.32 mg/liter allowed full bacterial action to go on.

4. To attain the toxic level of soluble copper, it was necessary to add enough copper sulphate to precipitate all of the sulphide and provide excess copper. In the present experiments, this required from 0.25 to 0.29 percent copper sulphate.

5. Desulfovibrio is enveloped in a zone of sulphide of its own making which protects it from all but massive doses of copper.

6. Anaerobic zones in sedimentary basins that are large enough to be useful in postulating biogenic precipitation of sulphide ores are also large enough to accomodate large influxes of dissolved copper without cessation or diminution of bacterial sulphate-reduction.

7. Copper toxicity is no argument against syngenetic theories of ore formation.

ACKNOWLEDGEMENT

This study was part of a joint research project of the Commonwealth Scientific and Industrial Research Organization and the Bureau of Mineral Resources carried out at the laboratories of the Division of Plant Industry, C.S.I.R.O., and supported also by the Australian Mineral Industries Research Association. This research project was organized in order to continue the work on geobiology that the late L.G.M. Baas Becking (1) had been carrying out in Canberra prior to the commencement of his last illness. Dr. D.O. Zimmerman of the Bureau of Mineral Resources and Mr. Keith Johns of the South Australia Geology Department were most helpful in conducting a field trip to Pernatty Lagoon.

REFERENCES

1. BAAS BECKING, L.C.M., and MOORE, D., 1961 - Biogenic Sulphides Econ. Geol., v.56, p.259-272.

2. DAVIDSON, C.F., 1962 - The origin of some strata-bound sulphide ore deposits: Econ.Geol.,v. 57, p.265-274.

3. DAVIDSON, C.F., 1962-63 - Discussion of paper by Brandt,R.T. Relationship of mineralization to sedimentation at Mufulira, Northern Rhodesia: Inst.Min.and Met.Trans., v. 72, p. 191-208.

4. SANDELL, E.B., 1959 - Colorimetric Determination of Traces of Metals: Interscience Publishers, Inc., 3rd Edition, p. 452-453.

Table I - Sulphur Distribution

Item	Flask No. and grams S				
	1	2	3	4	5
Copper sulphide - S	0.0545	none	0.0422	0.0507	0.0490
Dissolved sulphate - S	0.0649	0.0005	0.0126	0.0876	0.0239
Dissolved sulphide - S	0.0013	0.0486	0.0288	0.0001	0.0138
Volatile sulphide - S	0.0006	0.0009	-	0.0000	0.0027
Total S found	0.1213	0.0500	0.0836	0.1384	0.0894
Total sulphate - S reduced	0.0564	0.0495	0.0710	0.0508	0.0655
Percent of sulphate reduced	46.5	98.9	85.0	36.8	73.3

Table II - Copper Distribution

	Flask No. and grams Cu				
Item	1	2	3	4	5
Cu added as $CuSO_4 \cdot 5H_2O$	0.15890	none	0.06354	0.19060	0.08256
Copper sulphide - Cu	0.14400	--	0.08685	0.15680	0.09175
Dissolved Cu	0.00004	--	<0.00004	0.00075	0.00008
Other Cu*	0.00171	--	0.00385	0.02000	0.00260
grams $CuSO_4 \cdot 5H_2O$ added	0.6242	none	0.2496	0.7490	0.3244
percent $CuSO_4 \cdot 5H_2O$	0.249	0	0.099	0.289	0.129
molarity $CuSO_4 \cdot 5H_2O$	1×10^{-2}	0	4×10^{-3}	1.2×10^{-2}	5.2×10^{-3}

* Copper on walls of flask, in stopcock, or coprecipitated during analysis of sulphate.

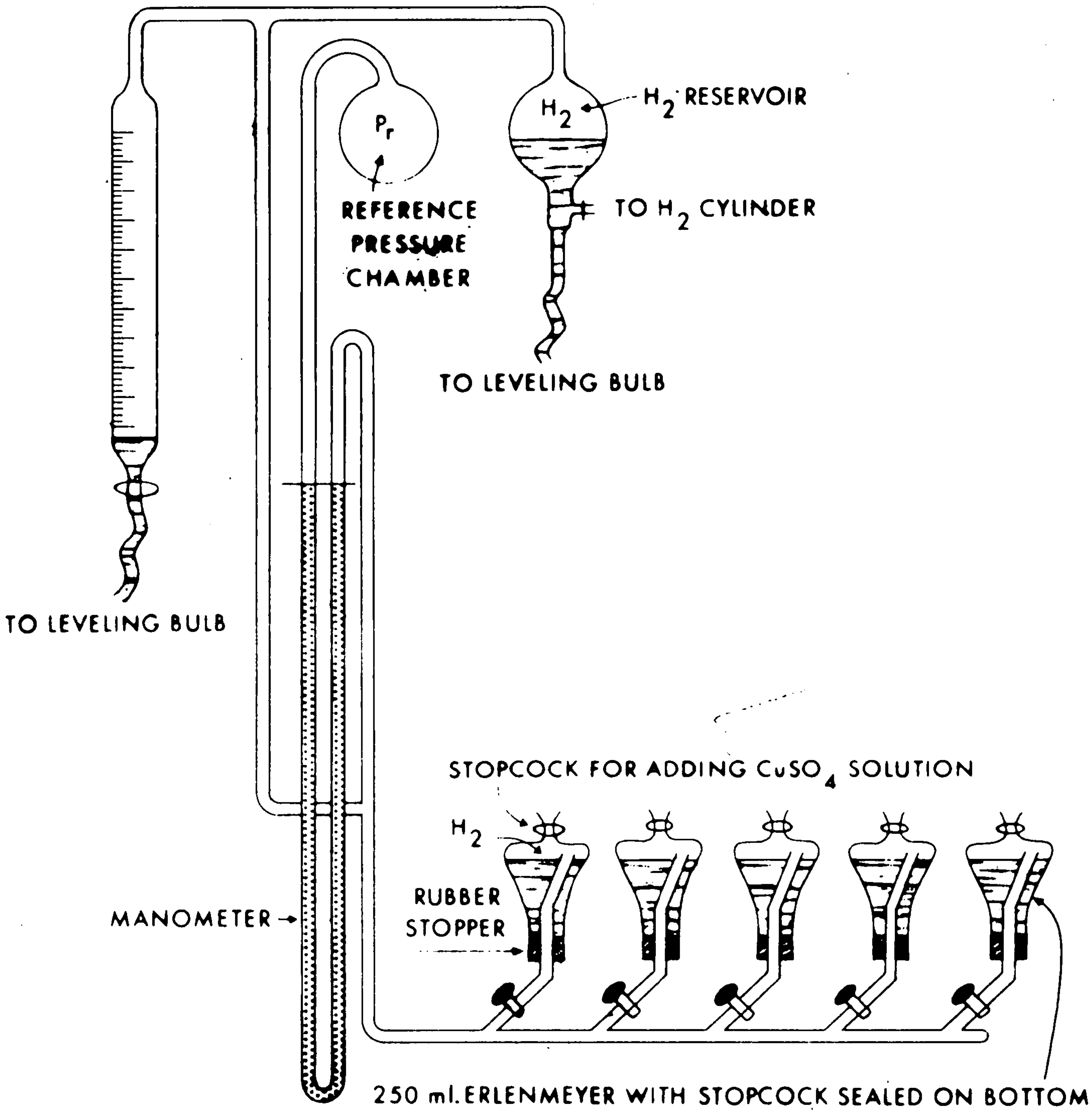

Fig. 1 - H_2 uptake measurement apparatus

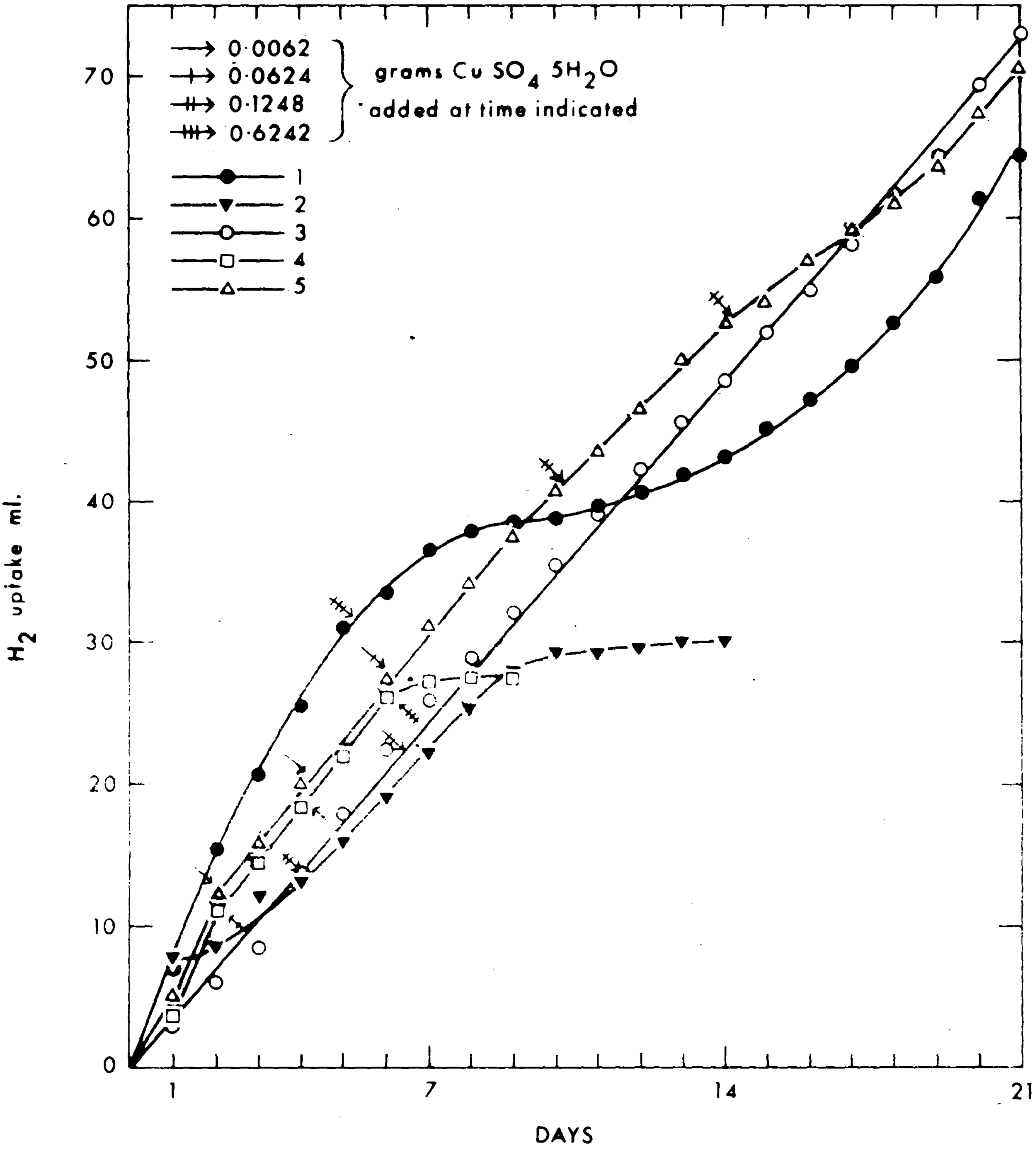

Fig. 2 - H_2 uptake and $CuSO_4$ addition

To accompany Record 1963/169.

M(E)/4